TERMINAL REPORT, CONTRACT NAS 9-8246

on

EFFECTS OF PROGRAMMED EXERCISE ON SKELETAL DENSITY AND CALCIUM BALANCE DURING HORIZONTAL BED REST OF HEALTHY ADULT HUMAN MALES

from the

TEXAS WOMAN'S UNIVERSITY

to the

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MANNED SPACECRAFT CENTER

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PREFACE

This study had as its major objective the effect of regular exercise, supervised according to a plan, on the bone density and calcium balance of healthy human males, as noted in the title of the project. In the protocol, however, other tests were made on blood and urine, with monthly reports covering many of these. For example, excretion of calcium and phosphorus have been covered in the monthly report dated 19 December, 1968. In this report, it was shown that urinary excretion, both of calcium and phosphorus, was higher during Bed Rest I, with no exercise practiced, than that during the ambulatory periods, or during Bed Rest II with regular supervised exercise. The monthly report dated 19 January, 1969, carried these comparisons further, giving the statistical differences between these periods, and showing further that the ones who exercised regularly excreted less calcium and phosphorus than those who did not exercise regularly. The bone density values also supported this finding.

Bone density and calcium - phosphorus excretion values during this study are given in full detail in the body of this report. Other analytical results have been given in some detail in monthly reports, and will only be reviewed briefly in this PREFACE.

Nitrogen. In addition to a further discussion of calcium and phosphorus, the monthly report of 19 February, 1969, contains a discussion, together with data, on the metabolic balance of nitrogen. The mean daily nitrogen balances were found to be positive for all of the periods of the study, although it was shown in the report of 19 March, 1969, that more nitrogen was excreted during Bed Rest I with no exercise taken than during the Pre-Bed Rest Ambulatory Period with a highly significant difference ($P < 0.001$). During Bed Rest II, however, those who exercised regularly excreted a slightly higher level of nitrogen than they did in the initial ambulatory, although the difference was only slightly significant($P < 0.05$).

Of those who exercised "at will", there was a highly significant difference between the quantity of nitrogen excreted during the preliminary ambulatory period than during Bed Rest II, with the latter higher than the former ($P < 0.001$). When Bed Rest I was compared with Bed Rest II, on the other hand, there was no statistically significant difference between the two periods.

Creatine and Creatinine. The report of 19 April, 1969, gave in some detail the urinary excretion of creatine and creatinine. Because of the importance of this phase of the study, the monthly report is reproduced in the Appendix.

Circadian Rhythm Phenomenon. As one of the important and interesting aspects of the Bed Rest Study under discussion, Circadian Rhythms were sought for various urinary components. This factor was discussed in the monthly reports for 19 May, 1969, and 19 June, 1969. The first of these issues includes brief reports found for rhythms which were plotted for calcium, phosphorus, nitrogen, creatinine and creatine, while the second covers various aspects of the urinary excretion of 17 - hydroxycorticosteroids during bed rest and ambulation, as well as the rhythms which were found in urinary excretions of this type of component. Copies of these two monthly reports also are given in the Appendix.

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INTRODUCTION

Man has pursued an evasive frontier over continents, across the seas, and to the poles both north and south. Man has looked to the ocean's depth and speculated as to the earth's core. Man's earliest records attest to his interest and concern in the space above him - the sun, the stars, the moon.

With the recent efforts on the part of scientists associated with the National Aeronautics and Space Administration, it has become possible for man to extend his stride from the earth to the moon and even to make much further inroads into space. No one knows at present what still may be in store for the inquisitive man who desires to explore the totality of the universe.

The Research Institute of the Texas Woman's University since 1963 has been engaged in research projects which contribute support to man's exploration of outer space. The study comprising this report is a part of a larger program conducted for the purpose of finding possible methods of maintaining the metabolic equilibrium and of conserving the muscular-skeletal integrity of adult male subjects during periods of horizontal bed rest, which has been shown to be the nearest to null gravity possible to attain on earth.

Bed rest studies in which these laboratories have participated have been reported by Mack and LaChance (1), by Mack and Creamer (2), and by Vogt, Mack, Beasley, Spencer, Cardus, and Valbonna (3). Evaluation of the bone density changes experienced by the astronauts of Gemini missions IV, V, and VII have been described by Mack, LaChance, Vose, and Vogt (4). Measurements of bone density changes during Apollo missions VII and VIII have been reported by Mack et al. (5).

In all bed rest studies and in all space missions investigated to date, skeletal density has been altered negatively in the os calcis, in the hand phalanges, in the wrist, and in the distal ends of long bones of the arm - the anatomic sites which have been investigated. The extent of the losses have been related to the dietary intake, particularly to the mineral content of the diet, and probably to other factors, which are not readily measured such as stress.

During the Gemini missions a new variable was introduced involving programmed exercise, primarily of the feet and legs (4). This exercise was made possible because of the fact that an exerciser developed by Dietlein and Rapp (6) was placed aboard the spacecraft during this flight for the purpose of measuring inflight exercise work tolerance.

The bone density losses experienced by the astronauts of the Gemini VII flight were far less than those of the astronauts of the Gemini IV and the Gemini V missions. Because marked differences in dietary intake between the astronauts of Gemini VII in comparison and that of the two earlier missions were found, with the Gemini VII men consuming most of the food put aboard for them whereas those on the two earlier orbital flights consumed only parts of their provided food, it seemed necessary to perform a supporting bed rest study in which exercise was the only variable factor. The results as described by Mack et al. (4) showed that, when food intake did not vary throughout the study, the lesser reductions in bone density were associated statistically with the exercise program.

In preparation for the Apollo missions, this laboratory was requested by the National Aeronautics and Space Administration, Manned Spacecraft Center, to conduct an investigation in which six healthy adult males would participate, with all variables controlled including diet. The broad objectives of the study were to determine the effects of programmed exercise during highly disciplined horizontal bed rest, with two 28 day bed rest periods conducted. During the first bed rest period no exercise was to be taken by any subject. During the second recumbency period, with an equilibration ambulatory period intervening, three of the subjects were to engage in the exercise program routinely four times daily, while three subjects followed the exercise plan under supervision, only when they would desire to do so.

The general objective of the investigation was to study means of maintaining metabolic equilibrium during periods of supreme inactivity, and of conserving simultaneously the muscular-skeletal integrity of the body.

The specific objectives of the study reported in this dissertation were the following:

1. To contribute to the total data amassed during previous studies by increasing the length of time followed and number of men employed under bed rest conditions;

2. To accumulate additional basic information on the relation of daily calcium intake to the maintenance of the integrity of skeletal mineral;

3. To include exercise of the hand and lower arm in a more direct manner than had been done previously by the use of a Hand-Gripper;

4. To include both isometric and isotonic types of exercise in the program;

5. To test the effectiveness of the Exer-Genie in reducing skeletal loss in the lower extremities during the recumbency of 28 days of bed rest;

6. To gain some insight into the amount of exercise which would produce an optimum benefit, by comparing the effects of varying levels of exercise in which the voluntary participants engaged in comparison with the fixed load of those taking part in the regular programmed exercise.

REVIEW OF LITERATURE

EARLY REPORTS ON EFFECTS OF DISUSE OF BONE

One of the first detailed reports of the effects of disuse of bone was made by Allison and Brooks (7) in 1921. In 13 experiments, dogs were employed with non-use of the forelimb achieved in three ways: (a) paralysis by section of the brachial plexus, (b) a flail joint achieved by excision of the upper end of the humerus, and (c) fixation by plaster-of-Paris cast.

Bone atrophy appeared to reflect the degree of non-use. The immobilized bone was thinner and lighter, with diminished breaking strength due to loss of bone substance. There was no change in the chemical composition of the bone.

Following their earlier report on bone atrophy, Allison and Brooks documented clinical cases of disuse of various types. They observed the changes in bone which result from lack of use of extremities in various clinical conditions and compared these observations with those made on experimental animals. The following groups of cases were studied: (a) poliomyelitis; (b) spastic paralysis; (c) peripheral nerve injuries; (d) Friedreich's atoxia; (e) pseudomuscular atrophy; (f) syringomyelia; (g) chronic infectious arthritis; (h) tuberculosis of bone; (i) acute pyogenic osteomyelitis; (j) ischemic contracture; (k) fracture; (l) congenital deformities; and (m) astasia abasia (8).

Whan a large number of persons were available in any of the groups, cases were selected so as to include various ages and periods of existence of the disabling disease. In all cases, the age, the duration of the disability, and the extent of the disability were obtained. When the lack of use was unilateral, the bones both of the used and nonused extremities were studied. Roentgen-ray examination was used to study the bones visually, with microscopic examination possible in some cases. The bones were studied as to length, thickness, diameter of the medullary canal, thickness of the cortex of the shaft and the number and thickness of the trabeculae of the cancellous bone. In children the epiphyseal cartilages were compared as to regularity and thickness. In making the roentgenograms, care was taken to have the bones as nearly as possible in the same position and at the same distance between the tube and plate, so that accurate comparisons could be made.

From the study of the changes in bones which followed immobilizations and lack of use from various causes, both in children or in adults, these authors found that the failure to use the bones was the cause of the bone changes, rather than the debility which preceded the cessation of use. There was no evidence that bone atrophy was the result of a specific nerve influence on bone.

One of the cases observed by Allison and Brooks and included in their report was a white woman 48 years of age, who was confined to bed for a period of a year incidental to repeated operations for an intra-abdominal disease. In this woman, all of the bones of the ex-extremities showed marked atrophy. The fact that bone atrophy occurs as a result of confinement to bed was noted by these investigators as a matter of special clinical significance at the time of the report.

The first experimental study of inactivity in humans was reported in 1929 by Cuthbertson (9), who thought that atrophy of muscle was of two distinct types---one which is mainly of reflex origin, and one which probably is entirely due to imposed rest. The problems were found to be complicated by an atrophy of bone. The amount of bony change varied with the degree of disuse, the cause of the disuse being immaterial.

Cuthbertson's subjects were volunteers in perfect health, or patients admitted to the hospital with loose fragments of cartilage in the knee-joint, but otherwise in good health. They were allowed to select the quality and quantity of foodstuffs which they desired, from a prepared list. This intake, as well as the intake of water, was kept constant during the course of the experiment.

The subjects were in nitrogen equilibrium. Within a day or two from the commencement of a period of muscle rest of the order described, a rise was noted in the excretion of sulphur, nitrogen, phosphorus, and calcium, in that order of priority. This loss was maintained fairly steadily for a varying period, after which it gradually declined.

Calcium equilibrium took longer to attain than Phosphorous. It was not attained in three of the experiments until the period of inactivity was well advanced. There appeared to be a retention up to that point. Whether this was due to the high calcium content of the diet, or not, cannot definitely be stated. There appears to be a tendency for a loss of calcium to occur as the period of rest lengthens. The animal experiments of Allison and Brooks (7) had previously demonstrated by visual examination of X-rays and by breaking strength of bones that bone atrophy through disuse could take place readily. Cuthbertson (9) concluded that experiments dealing with metabolism of phosphorus and calcium should be of much longer duration, and, that they should have much longer pre-periods than those dealing with the metabolism of nitrogen and sulfur.

It is of interest to note when reviewing a report by one who is regarded to be the first investigator in a certain field to see whether or not he cites earlier investigators as preceding his work. A case in point is that Cuthbertson cited two persons as having worked in his field previous to his early report. They were Beigel (10), and Shaffer (11), with neither of them having made a formal publication of their observations.

Beigel had conducted a brief study in 1855 in which he had compared the excretions of four healthy subjects during a period of active recreation with a period of rest on a sofa. He had found differences in the mean quantity of urea excreted per day, with the excretions greater during the period of activity. He had not included calcium or other bone minerals in his limited experiment, nor had he related his findings to bone changes. His experiment had no controls, nor had he explained the lengths of time of his observations.

The observations of Shaffer were made in 1908 on a man who spent two days wholly in bed, and then for the following four days was up for a few hours to sit in a chair. This period was followed by one of normal activity. The average total daily nitrogen excretion was 4.77 g. and 4.40 g., and the sulphur excretion was 0.438 g. and 0.424 g. during the inactive and active periods, respectively. Shaffer concluded that either increase or decrease of muscular activity within physiological limits has per se no effect on protein metabolism as indicated by the urinary excretion of nitrogen and sulfur. Cuthbertson noted, however, that it appears that no pre-test period was followed before the experiment began to insure that nitrogen equilibrium had been established. In addition, the inactivity period was short and without control.

In 1941 clinical interest concerning the possible effects of prolonged bed rest on human subjects was aroused by the study of Albright, Burnett, Cope and Persons (12) of Harvard in which a 14-year old boy who suffered from a fractured femur caused by a bone cyst was immobilized because of the injury. The patient developed marked hypercalcemia and hypercalcinuria and he was examined for a possible parathyroid tumor without success.

Albright and his co-workers thereupon concluded that immobilization of a large part of the skeleton in individuals may result in hypercalcemia associated with a rapidly developing osteoporosis. The patient was mobilized and promptly exhibited improvement.

In 1945 Howard and Bigham of Johns Hopkins (13) studied the urinary excretion of calcium and phosphorus in patients convalescing from fracture. The subjects were 13 male patients aged 14 to 64 years. These investigators studied the pattern of urinary excretion of calcium and phosphorus in patients immobilized in extensive casts after fracture of the femur. the tibia, and after femoral osteotomy.

Dietary content of the food was calculated from Sherman with some check analyses in the investigator's laboratory. Figures used for milk were determined by analyses.

Abrupt changes in calcium of the diet from high to low or reverse were made.

The caloric and nitrogen ratios of the diets were approximately 1000 calories per 6.25 grams, so that the caloric content could readily be ascertained from the amount of ingested nitrogen.

The sustained excretion of calcium via the urine was but little influenced by dietary changes, or by administration of calciferol or alkalis. It was similar to that found in patients on constant diets. Giving calcium in milk (with coincident changes in phosphorus) did not show significant changes in urinary calcium; but when a large increase in dietary calcium was provided as calcium lactate, given in water after meals and then withdrawn, somewhat larger changes in urinary excretion were noted.

Even on fixed constant diets of all types used in these experiments, the daily output of these elements in the urine fluctuated to some extent (up to 100 mg. or even more), due to other influences as yet not determined. Therefore, a dietary change must uniformly affect an alteration of more than 100 mg. per day in the urinary calcium or phosphorus output to be considered significant.

Roetgenographic studies of limbs encased in plaster or in splints for long periods of immobilization usually disclose marked rarefaction of the bones. The movement of calcium and phosphorus, with especial attention to the amounts and concentrations of these elements in the urine, was studied in a group of 17 patients at bed rest with complete leg and partial body casts.

In order to evaluate the relationship between the amount of ingested calcium and the calcium content of the urine, abrupt changes in the calcium of the diet from high to low or in reverse order were made in several instances.

A research project was reported from the Laboratory of Physiological Hygiene, University of Minnesota in 1945 on the effects of three weeks of complete bed rest on blood volume and related measurements. This study was reported by Taylor, Erickson, Henschel, and Keys (14).

A second study from the same laboratory was published in 1948 by Taylor, Henschel, Brozek, and Keys (15). These investigators used six young men as subjects in preconditioning, bed rest, and reconditioning periods. The men were kept in a precondition period of six weeks followed by three weeks of bed rest and six weeks of reconditioning. During the initial conditioning period they walked on a treadmill under standard conditions for six days a week. A training period in the psychomotor area also was included in the program. Cardiovascular response to posture was determined by means of tilting measurements.

The diet was adjusted to maintain cardiac balance as estimated from body weight. Pulse rates, respiratory efficiency, oxygen consumption, respiratory quotient, and mechanical efficiency were measured before and after bed rest.

Bed rest produced a 17 per cent decrease in heart volume and a decrease in the transverse diameter of the heart. Pulse rate, oxygen consumption, and other tests changed as a result of bed rest. Bed rest produced a marked deterioration in cardiovascular response as measured by pulse rate and blood pressure changes produced by tilting to 68° on a tilt table.

Three reports from the Department of Medicine, Cornell University Medical College, The New York Hospital and The Russell Sage Institute of Pathology have been published on the interrelationship of immobilization of healthy subjects with various metabolic factors. The first of these reports was published in 1948 by Dietrick, Whedon, and Shorr (16). This constituted a classic contribution to the understanding of many of the effects of bed rest recumbency.

This investigation included four healthy young men, of whom two were participants for a period of six weeks and two for seven weeks.

These subjects were continued in an initial conditioning phase for periods of six to eight weeks. During this control period they engaged in exercise of many types. During the bed rest period the subjects were placed in bivalve casts which extended from the umbilicus to the toes. The men were removed from the casts each day for hygiene purposes and for tilt table and ergometer tests.

The men were on a constant diet which provided 2,500 to 2,800 calories, 85 to 90 grams of protein, 0.852 to 0.920 grams of calcium, and 1.50 to 1.64 grams of phosphorus. Dietary analyses were carried out during the course of the studies. The biochemical tests included a series of hematological measurements, blood tests for calcium, phosphorus, and total nitrogen, urinary analyses for pH, volume, calcium, phosphorus, total nitrogen, creatinine, creatine, sulfur, citric acid, sodium, and potassium, fecal analyses for calcium, phosphorus, total nitrogen, sodium, and potassium, and food analyses for calcium, phosphorus, total nitrogen, sodium, and potassium.

X-rays were made of the long bones and spine under duplicated X-ray techniques in an effort to detect visually discernible bone rarefaction resulting from immobilization.

Compared to the pre-immobilization tests, there was a decrease in total blood volume and a marked deterioration in exercise tolerance after immobilization, with most of the physiological functions showing recovery from three to six weeks post-immobilization.

With respect to the metabolic findings, immobilization brought about a prompt increase both in urinary and fecal calcium. Calcium excretion (urinary and fecal) recovered slowly and continued to show values greater than the control period during the first three weeks. Minimum post-immobilization calcium levels were reached in two subjects during the fifth and sixth weeks, with a longer time required for the other two subjects.

In the second study of this series, Whedon, Dietrick, and Shorr (17) became concerned with measures which might reduce the disadvantageous results of long immobilization. This investigation involved an investigation of the Sanders slowly oscillating bed. Three normal healthy young men who were studied with the earlier group, who had been on a constant dietary intake before, during, and following a five-week period of immobilization. The men were maintained in the same type of plaster casts used in the former study, with the oscillating beds used during the recumbency period. The three subjects had taken part in the immobilization experiment on standard fixed beds, and hence the metabolic and physiologic data were available from the former experiment, and could serve as a control for this study.

During the control periods the subjects were approximately in calcium equilibrium. During the immobilization phase, there was a gradual increase in calcium excretion, primarily in the urine. The increase in fecal calcium was comparatively small. Urinary calcium increased less rapidly than in the fixed bed experiment. For all three subjects the maximum urinary excretion for a three- to four-day pooled calcium sample had an average of 311 mg. This is to be contrasted with a higher urinary calcium excretion by the same three subjects in the fixed bed experiment. During the recovery phase following immobilization in the oscillating bed, calcium excretion decreased toward control levels more rapidly than it had in the fixed bed study.

Phosphorus and nitrogen excretion levels followed somewhat the same pattern as calcium, especially in two of the subjects. During the recovery phase, most metabolic and physiologic functions returned to normal more rapidly following the oscillating than following the fixed bed experiments.

The third study of this New York series was carried out by Whedon and Shorr (18). This involved a consideration of a seriously immobilizing disease, acute anterior poliomyelitis. Altogether 11 subjects were studied, nine of them in a metabolism ward where they received constant dietary intakes for periods which ranged from five to nine months. Seven of the nine patients had all four of the extremities involved.

The following tests were made during this experiment: urinary and fecal calcium; urinary and fecal phosphorus; urinary citric acid; serum calcium and phosphorus; and serum alkaline phosphatase.

The first mobilization to a chair was 2 1/2 months, with walking beginning 3 1/2 months after onset. The average period before walking could be begun was six months for seven of the patients. The other two progressed only to a wheel chair while the study was in progress.

Correlation between serum calcium levels was high for total calcium loss, maximal negative calcium balance, and maximal urinary calcium excretion. Maximal negative calcium balance occurred on the average at nine weeks after onset. Negative calcium balance continued for an average of seven months, with positive balance regained approximately after patients began to stand on their feet.

Metabolic studies were made by Lynch and colleagues (19) on 44 healthy men before and during bed rest at ground level and at a simulated altitude of 10,000, and 12,000 feet. Simple bed rest resulted in loss of calcium, phosphorus, nitrogen, sodium, and chloride with little change in potassium. The simulated altitude of 12,000 feet significantly reduced the loss of urinary calcium, phosphorus, nitrogen, sodium, chloride and total nitrogen. The 10,000 foot simulated altitude was associated with urinary calcium losses quantitatively intermediate between ground level and 12,000 feet, with increased total sodium and potassium losses. The possibility that bone resorption represents one aspect of acclimatization to simulated altitude was discussed in this report.

Birkhead, Blizzard, Daly, Haupt, Issekutz, Myers, and Rodahl (20) at Lankenau Hospital, Philadelphia, in a project monitored by LaChance at Wright-Patterson Air Force Base made an extensive study on the effect of bed rest on four healthy men. The men were admitted to the metabolic ward of the hospital three weeks before the beginning of 40 days of bed rest. During the preliminary period they were given a program of physical training. They were retained for three weeks following the bed rest phase of the study for observation and physical status improvement.

During the study the subjects were on a weighed diet of 2,523 calories, 72 grams of protein, 74 grams of fat, 385 grams of carbohydrate, and 1.724 grams of calcium. Analyses of the diet for calcium and nitrogen were made at frequent intervals. The diet consisted primarily of a liquid formula. During the bed rest the only activity allowed the subjects was the movement of arms and legs.

In addition to extensive functional and physiological tests, the following metabolic analyses were made: blood tests for sodium, potassium, chloride, hemoglobin, hematocrit, alkaline phosphatase, calcium, cholesterol, phospholipids, and inorganic phosphorus; urinary tests for excretion of nitrogen, of calcium and of phosphorus; and fecal tests for nitrogen.

Metabolic findings from this investigation include the following. All four subjects were in nitrogen balance throughout the study. This is at variance with certain other investigators, although the finding is discussed by the authors. The most marked changes found during bed rest included definite increases in excretion of calcium and phosphorus. In some cases the urinary excretion of calcium more than doubled, and the increase began during the first six days in bed.

Hemoglobin and hematocrit values decreased in the course of the experiment, and reached their lowest levels at the end of the recovery phase. No other remarkable blood value changes were noted.

OTHER ASPECTS OF RECUMBENCY

As early as 1944 numerous investigators published reports on the abuse of rest as a therapeutic measure for patients with cardiovascular disease. Interest in this subject had been stimulated by visits to a number of European clinics where physicians had observed that patients with heart disease were allowed to sit up in chairs and to walk about the hospital wards. This point of view concerning the treatment of heart disease was expressed by Levine (21). During the same year Eastman (22) published an article on the abuse of rest in obstetrics.

Immobilization of human subjects has been reported by numerous investigators as causing an increase in whole blood volume, including the red blood cell volume and the serum volume. This has been reviewed by Oberfield et al. (23), who have described in detail their method of blood studies by the chromium 51 technique.

Issekutz et al. (24) have investigated the effect of prolonged bed rest on urinary calcium output. They state that it has long been known that prolonged inactivity in the horizontal position increased the urinary excretion of calcium in healthy young men, with the nitrogen output not showing any consistent response.

Heaney (25) has reported on radiocalcium metabolism in disuse osteoporosis in man. This investigator states that metabolism is dependent upon mechanical factors, such as muscle pulls and weight bearing. When these factors are decreased or removed, bone mass decreases and osteoporosis ensues. Combined calcium balance and radioactive turnover studies have been performed in a series of normal and paralyzed adults. The kinetic factors responsible for disuse osteoporosis were discussed in this report.

The book by Davidson et al. (26) on Human Nutrition and Dietetics states that disuse atrophy, which is a common occurrence in bedridden patients, may be related to reduced physical activity.

Lutwak and Whedon (27) have described osteoporosis as one of the most common, yet least understood afflictions of bone. Disturbances in protein, mineral, or hormonal aspects of bone metabolism have been implicated as causative factors which lead to increased resorption of bone. Osteoporosis is described by these authors clinically, chemically, radiologically, and histologically. No single therapeutic approach has seemed to be effective in all osteoporotic patients. The use of hormones, Vitamin D, and dietary calcium needs to be examined, according to these research workers, and a multifaceted approach was recommended.

Kottke (28) has reported on the effect of limitation of activity upon the human body. This investigator states that, although rest may be protective for a damaged organ, it results in progressive loss of functional capacity for normal organs. Limitation of activity during prolonged bed rest causes significant deterioration of multiple organ systems, including the skeletal, neuromuscular, cardiovascular, respiratory, urogenital, and nervous systems. Neuromuscular and emotional control and intellectual performance are all impaired by prolonged bed rest.

Kosmolinsky and Dushkov (29) conducted an investigation related to specific features of adaptation of human subjects to prolonged stay in sealed chambers. A group of men aged 24 to 38 years took part in the experiments in which they were placed in sealed chambers of small volume in which the temperature was maintained within 20 to 25 degrees Centigrade, with normal atmospheric pressure.

No pathological deviations were observed in the psychologic activity of the tested subjects.

Changes in the nerve-emotional sphere testify to some reduction in emotional stability. Pronounced changes in hormonal metabolism indicate the stress-condition. The changes in higher nervous activity indicate a small reduction of the excitation process in the central nervous system.

At the beginning of prolonged experiments mental performance sharply deteriorates, then on the 9th-10th day it is stabilized. At the end of the experiment under the effect of environmental factors (sensory deprivation, low motoricity, monotonous pose) mental performance again diminishes. There is a definite dependence on volitional processes (the so-called "final effort", i.e. the improvement of indexes before the discontinuation of the experiment).

As a rule, muscular performance gradually goes down from the first to the last day of the experiment, increasing only before leaving the chamber.

EXERCISE AND PHYSICAL STATUS

Vogt, Mack, Beasley et al. (3) conducted the first bed rest study during which bone density measurements were made and calibrated exercise was added to the investigation while the subjects were in supine, horizontal bed rest. The experimental study was performed at the Texas Institute for Rehabilitation and Research with the cooperation of the Texas Woman's University in the Summer of 1963 to evaluate (a) the changes in os calcis bone density with bed rest and (b) the effect of isometric exercises in preventing the loss of calcium during prolonged bed rest.

Seven healthy young adult males participated in the study which was divided into two 14-day periods of bed rest, preceded and followed by an observation period. The first bed rest period consisted of bed rest without exercise. Bone densitometry measurements of the os calcis showed a diminished density with the institution of bed rest, the greatest changes occurring in the first 3 or 4 days.

During the second immobilization period, the subjects were required to perform controlled isometric exercises at hourly intervals beginning at 9 a.m. and ending at 4:30 p.m. The exercise procedure was designed to provide the following:

1. Musculoskeletal exercise on the os calcis and lower back, since x-ray densitometric studies were used to evaluate the os calcis and third lumbar vertebrae. Previous experience has been accumulated on radiographic analysis of these anatomical sites.

2. Exercise to massive muscle groups of the lower extremities, back, and shoulders, since it was thought that lack of exercise may contribute to the "cardiovascular deconditioning" of bed rest.

3. Relative gravitational constancy (i.e., action of gravity vectors on body does not change direction) as required in the design of the cardiovascular portion of the study.

Apparatus was constructed for use in bed to provide musculoskeletal exercise to the lower extremities and back with the body maintained in a horizontal position.

A recording was made of every exercise routine for each subject. An auxiliary meter, synchronized with the myodynagraph, was placed for easy viewing of the subject to allow him to maintain the desired forces, and thus perform the prescribed amount of exercise with each trial. The subjects were required to produce a thrust force in the range of 300 - 400 pounds, the desired value having been determined for each subject before immobilization. Maximal effort forces were variable for the different subjects, and the exercise levels prescribed were approximately 60 per cent of maximum for each individual.

When the bone mass data for the central section of the os calcis were pooled for the pre-bed rest period and were compared statistically with the pooled data for all roentgenograms made during the first bed rest period, the former surpassed the latter by a significant difference ($P < 0.01$) for all subjects grouped together. The differences also were significant for each individual subject, with $P < 0.10$ to $P < 0.001$ representing the range for the respective subjects.

The results obtained during the bed rest with exercise period showed bone mass values which exceeded those found in the same subjects during the first bed rest period when exercise was not administered. When the data were pooled for all subjects for each of the two periods, the bed rest with exercise bone mass surpassed that of the period without exercise significantly ($P < 0.05$). When the data for individual subjects were considered for the two respective periods, the same findings occurred, with $P < 0.10$ to $P < 0.001$ denoting the range of statistical significance found for the individual subjects.

The daily diet of the subjects was designed to approximate a 2,400-calorie, 1,000 milligram calcium, and 100 gram protein composition. The average calcium intake by analysis during the first study period was 1088 milligrams per day. During the second study period it was 1035 milligrams daily.

Van Liere and co-workers (30) found that exercise imposed upon laboratory animals caused a significant increase in their intestinal motility. In discussing their results, these authors stated, "Since exercise produces hypertrophy of striated muscle, it is conceivable that the muscles of the viscera, likewise, would be strengthened by increased work."

In an interview in 1959, Dr. A. L. Chapman (31), formerly of the United States Public Health Service, was asked: "Why is exercise helpful?" In replying, Dr. Chapman stated that blood vessels are lined with smooth muscle fibers which atrophy like all other muscles when they do not get exercised. He went on to explain that a demand on the blood stream for oxygen was the way to exercise a blood vessel. When muscle tissues use up oxygen at an increased rate, as in physical activity, the heart beats faster to renew oxygen-rich blood to the tissues. Blood vessels expand to allow this more profuse circulation.

Davis, Logan, and McKinney (32) list the following biophysical values as benefits derived from muscular activity:

1. Muscular activity stimulates growth and development.
2. Muscular activity improves the efficiency of the living organism.
3. Muscular activity increases adaptability to external forces.
4. Muscular activity aids in the body's internal adaptation.
5. Muscular activity promotes desirable emotional releases.
6. Muscular activity helps prevent bodily injury.
7. Muscular activity aids in skeletal atrophy restoration.

Matthias (33) wrote that the tendon, according to the extent of the muscle pull, transmits to the underlying bone a lesser or a greater action. These "pull-pressure effects" exerted on bone by muscle and tendon constitute a development stimulus on the bone. This stimulus makes itself felt in two directions: on the one hand, it activates a growth of bone in bulk and size; and on the other, it provokes a change of the inner structure, location, and number of bone fibers, in order to meet the varied demands of power exertion. Of great importance is the fact that there exist, in this connection, specific laws of stimuli effects. It has been shown that amount, as well as duration, of muscle function is of decided significance in bone mineral stimulation.

Brannon, Rochwood, and Potts (34) attempted to show that specific exercises could prevent debilitating musculoskeletal disorders which would be of interest in space travel.

The importance of regular activity and exercise to normal body function generally is conceded. Normal osteoblastic capacity depends to large measure upon the stresses of muscular contractions and weight-bearing compression forces. The loss of these stimuli results in insufficient formation of bone matrix, inadequate deposition of Ca salts, and increased porosity of bone. The softened bone loses its strength and resiliency and is subject to pathological fractures. The maintenance of the healthy state of striated muscle also requires the stimulus of gravitational stress and activity. Elimination of these factors produces hypotonicity and wasting of muscle.

The undesirable metabolic and physiological effects of lengthy confinement and inactivity on the musculoskeletal system are well documented. Clinical observations and experimental studies have demonstrated that muscle atrophy and bone demineralization readily occur under these circumstances, influenced by degree and duration of inactivity.

Subjects were 30 volunteer airmen aged 18 to 22, all healthy adult males. Environment, diet, activity, and health of participants were strictly controlled. All subjects were given the same 2300 calorie diet which included a daily average of 1.25 grams of calcium, 250 grams of carbohydrate, 100 grams of fat, and 106 grams of protein. Meals were served three times daily. The study lasted 60 days.

Three types of exercise were investigated, as follows: (a) the official U. S. Air Force physical fitness program which includes basic exercises of the calisthenic type, (b) Isotonic exercises, and (c) Isometric exercises. The men were divided into these five groups of six men each: Subjects in Category I were restricted to the ward, but were allowed normal activity as members of a work detail. Category II subjects were confined to bed rest and were given isotonic exercises with 10 pounds of applied resistance and 10 repetitions of each exercise. Those in Category III were confined to bed rest and given isometric exercises without applied resistance, and with 10 repetitions of each exercise. Category IV were confined to bed rest and were given isometric exercises using the resistance of the agonist muscle or the bed, with a count of six during each muscle contraction, with 10 repetitions. Category V subjects were confined to bed rest and given no exercise routine. Movements of those in the last group were confined to sitting, turning, eating, washing, handicraft projects, and use of the bed pan.

Laboratory tests included blood tests for: (a) hematological values; (b) blood values for calcium, phosphorus, alkaline phosphatase, potassium, sodium, creatine, creatinine, chloride, cholesterol, urea nitrogen, glucose, total protein, albumin, and A/G ratio. Urinary determinations included tests for: specific gravity, pH, calcium, potassium, sodium, chloride, creatine, and creatinine.

Body weight changes, physiological measurements, and tests of extremity muscle strength and mass also were included in the tests.

No abnormal alterations were found in the red cell blood count, hemoglobin, hematocrit, or prothrombin time. The daily urine outputs for each subject did not fluctuate appreciably for the 60-day period. Urine determinations made on the 24-hour total volume aliquots failed to show variations in the specific gravity, reaction, or chemical tests.

With respect to body weight, the Category I group (normal activity) gained an average of 1.4 kilograms during the study period, while the subjects in the bed rest categories lost in average weight. There was no loss of power in the hand grip of those in Category V (bed rest with some activity, but no routine exercise), although loss of girth in the forearm and upper arm averaged 0.7 cm. All bed rest categories showed some decrease in the girth and strength of the calf muscles, with this most evident in Category III (bed rest with isotonic exercise without weights) and Category V (bed rest with some activity, but no weights.

Elton (35) reported on stress reaction of bone in army trainees. The author had spent four years in the practice of orthopedics primarily with problems of the army trainee. The author states that the term "stress reaction" of bone indicates that a normal healthy adult male can suffer from such a reaction, which is regional in nature and which manifests itself by osteoporosis of bone. This is followed by breakdown of the internal trabecular bone pattern, and at times, but not always, by frank fracture of the bone at its weakest point.

Ganslen (36) has reported on the influence of training and exercise upon the physiological and mechanical properties of muscle. He states that, since 40 per cent of the human body is devoted to muscle, human motor performance should be considered in terms of "biomechanics" or "dynamic anthropometry".

This author recommends moderate exercise, and states that we should train our ligaments, cartilage, and tendons, as well as our muscles.

Stenberg et al. (37) of Stockholm, Sweden has reported on the hemodynamic response to work with different muscle groups, sitting and supine. This group has conducted experiments which included submaximal and maximal work on bicycle ergometers with arms, with legs, and with arms and legs, in sitting and supine positions, respectively. The lowest exercise values were registered during arm work in a sitting position, and the highest during arm and leg work in a supine position.

Bevegard et al. (38) also working in Sweden, have reported on the circulatory adaptation to arm and leg exercise in supine and sitting positions. In six healthy, young males, the adaptation to arm, leg, and combined arm and leg exercise was studied by cardiac catheterization in supine and sitting position. The hemodynamic and ventilatory responses were equal during leg exercise and when more muscle groups participated as during combined arm and leg exercise. During exercise with the arms, however, total ventilation, heart rate, and lactate formation were significantly higher for a given oxygen uptake. With arm exercise the systolic, diastolic, and mean pressures in the aorta increased more in relation to the cardiac output than when the legs participated in the work. The observed differences in circulatory adaptation during arm versus leg exercise indicate higher sympathetic tone during arm exercise. The effect of body position was more pronounced during arm exercise only than when the legs took part in the work. In the sitting position the stroke volume did not increase on transition from rest to arm exercise when the legs were passive.

Cooper (39) in discussing the physical effects of seated and supine exercise, with and without subatmospheric pressure applied to the lower body, states the following:

Eight subjects were evaluated on a bicycle ergometer once a week for four weeks in both the upright and the supine position, with and without the addition of -30 mm. mercury lower body negative pressure. Upright ergometry without negative pressure was associated with the highest maximum oxygen consumption, whereas upright exercise with negative pressure and supine exercise with and without negative pressure were remarkably comparable. The cardiovascular response during submaximal upright exercise with negative pressure resembled that seen after physical deconditioning. This difference was not as apparent at maximum performance. These results indicate that in an earth environment the integration of lower body negative pressure with upright exercise provides an overload phenomenon that may be used to accelerate a cardiovascular conditioning response. In space, the mechanics of exercise might be facilitated, a good cardiovascular conditioning device could be provided, and a means of orthostatic stress testing would be available.

Greenleaf et al. (40) made a study on the physical performance of women following heat-exercise hypohydration. In this study, 12 healthy women, ages 22 to 33, underwent a 5-month physical training period before being divided into two groups, a control group and a hypohydrated (water-depleted) group. Hypohydration was achieved with the subjects alternately resting and walking (4.8 km/hr) at 49 C until they lost about 3.3 per cent of their body weight. They were then given various physical performance tests to assess the effect of the hypohydration. Statistically significant changes ($P < 0.05$) in the hypohydrated group were observed in (a) resting pulse rates, (b) recovery pulse rates following a modified Harvard step test, and (c) the pulse rates and systolic and diastolic blood pressures during a standard 70° tilt table test. No significant decrements were noted in submaximal O_2 intakes, submaximal V_E, total body reaction times, and maximal isometric muscular strength. The submaximal ventilatory exchange ratio was unchanged. It was concluded that there was some deterioration in the cardiovascular system response but there was no gross deterioration in physical performance following 3.3 per cent hypohydration in fit, young women.

Wasserman et al. (41) have reported on the interaction of physiological mechanisms during exercise. The effects of work intensity and duration on the metabolic,circulatory and ventilatory response to exercise are quantitated in healthy male subjects during cycle ergometer exercise. There is a well-ordered relationship between work rate and oxidative energy sources. Steady-state time for oxygen consumption is dependent on work intensity (the time arterial blood lactate concentration stops increasing). At moderate work, anaerobic metabolism is a very small part of credit oxidation, while at very heavy work, the pyruvate-lactate mechanism is the major creditor. The increase in gas exchange ratio reflecting the production of carbon dioxide from bicarbonate (buffering of lactic aid) is transient. After the steady state is reached, the ratio decreases. The heart rate increase during constant-load exercise parallels the increase in oxygen consumption. Physiological dead space/tidal volume ratio decreases from .33 to .17 during exercise. The reduction is independent of work duration and only slightly reduced as work intensity increases. Arterial oxygen tension does not decrease during exercise at sea level even at maximal rates of oxygen transport. Arterial-end tidal carbon dioxide tension differences suggest a fluctuation in pulmonary capillary carbon dioxide tension of approximately 8 mm Hg during the respiratory cycle of the exercising subject. Changes in minute ventilation are best predicted from the rate of carbon dioxide production and the extent of respiratory compensation for metabolic acidosis.

Raab et al. (42) have reported on cardiac adrenergic preponderance due to lack of physical exercise and its pathogenic implications. The authors state that lack of exercise resulting from motorization, automation, television sitting, and from the nearly universal availability of the multitude of labor-saving devices, has become one of the most conspicuous characteristics of 20th century Western civilization. The study made by this group of experimenters involved 360 healthy American and Austrian men, 17 to 50 years of age.

The results, especially the basic resting values, reveal a linear increase of cardiac sympathetic tone and decline of cardiac neurovegetative counterregulatory effectiveness in proportion to decreasing degrees of habitual exercise. Physical training periods reversed the situation.

Expanded epidemiological studies of primary neurovegetative factors together with other correlated pathogenic conditions (dietary, social, emotional), and the development of medically supervised, prophylactic mass reconditioning programs for degenerating sedentary populations are being urged.

Wisham et al. (43) have discussed the value of exercise in peripheral arterial disease. These investigators conducted a study to ascertain whether or not exercise actually does produce an increased blood flow in the extremities of persons with and without peripheral arterial disease. It also was desired to determine quantitatively the changes in blood flow produced by a series of exercises requiring progressively greater muscular effort. The method used was that of measuring the clearance of radiosodium (Na^{24}) from tissues. The rate of this clearance reflects the effective circulation in these tissues. In this way the effects of exercise were determined both during and after exercise.

Because, in a normal person, blood flow is greatly increased in muscles exercised against resistance, the effectiveness of this form of therapy in peripheral arterial disease was also studied and compared with the more commonly used forms.

The conclusions reported by these research workers were the following:

1. Passive postural exercises do not increase blood flow significantly.

2. Active exercise is effective in increasing blood flow during and shortly after its administration.

3. The effect of exercise increases with increase in muscular effort.

4. The most effective form of exercise is that in which the muscles of the involved extremities are offered considerable resistance against their contraction.

Smith and Kidera (44) have conducted an experiment on the effect of programmed exercise on non-specific T-wave abnormalities. An abstract of this study follows.

"Fifty asympotomatic persons with non-specific T-wave changes were placed on a progressive exercise stress program. Six cases showed no essential change in the resting electrocardiogram or exercise test after a period of programmed exercise. Five of these cases were under 35 years of age and the changes were probably due to old, healed myocarditis-pericarditis from a previous infection. One case in a 45-year-old male also showed no changes on exercise. Forty-four cases showed rapid changes in the T-waves, returning to normal within one to three months. Rapid progression of exercise stress was well tolerated and no untoward symptoms developed.

It is probable that the abnormal T-wave changes in the group over 35 were due to early myocardial ischemia and hypokinetic disease and emotional reactivity. The use of progressive exercise stress seems to benefit all of these factors and the return of the electrocardiogram to normal is an indication of these benefits."

General interest in physical activity is becoming widespread. An International Symposium on Physical Activity and Cardiovascular Health (45) was held in Toronto, Ontario, Canada from October 11 to 13, 1966, sponsored by the Ontario Heart Foundation, the Ontario Medical Association, and the Canadian Medical Association. The concluding remarks made at the Symposium, made by P. O. Astrand, M.D. of Stockholm, Sweden included the following:

> "In regard to the prediction of aerobic capacity, the problems are still unsolved and there will yet be many attempts to solve them. If you have a research interest in the maximum oxygen intake you should measure this quantity. If you are merely seeking a practical screening test, you should ask yourself what accuracy is necessary for your purpose. The heart rate may be

influenced by factors other than oxygen transport. I could take as an example the downhill skier. When the skier is about to start, his heart rate as measured by telemetry is about 150 beats per minute, and it has been so for the previous minute; within seconds of take-off, his heart rate will reach his maximum—about 200. I refer to measurements on one of the best downhill skiers in the world. His heart rate stays at 200 throughout his descent. We have also measured his oxygen uptake, and a heart rate of 180 would be adequate to transport the amount of oxygen he needs, so that the additional rate must be due to psychological factors. We have used a similar telemetric system to record the heart rate of a girl attending a concert given by the Beatles. When the theatre orchestra was playing, her heart rate varied between 120 and 150. When they announced that the Beatles were approaching, her heart rate increased from about 100 to 160; when they appeared it reached 200, and it stayed at this level, although I would presume that her oxygen uptake and cardiac output were low. The prediction of maximum aerobic capacity from such data would give erroneous results! We have also noticed during telemetric studies of car drivers that invariably, if there is a bend in the road that the driver does not expect, the heart rate increases by about 20 beats/minute or more.

"The strain on the individual may be related more to the heart rate than to the oxygen uptake. My wife has measured both the maximum heart rate and the average heart rate of the housewife while working around the house; she has also made similar measurements for other professions, and found that the mean heart rate over an eight-hour day is related to the maximum heart rate. Individuals with a maximum of 200 work at a heart rate of about 115, while those with a maximum of 150 work at a heart rate of about 90. You cannot use the heart rate alone to tell how hard an individual should train. If your goal is a heart rate of 180, this will be discouraging for the person with a maximum heart rate of 150! The pulse rate selected should be related to the individual's maximum. In this further discussion of prediction methods, I again want to emphasize that if you plot data for different ages from 20 to 60, the heart rate at a given work load stays the same. Conversely, the work load at a heart rate of 170 is more or less the same throughout the span of adult life. The heart volume and total hemoglobin also remain about the same, and the maximum Vo_2 is probably influenced mainly by the decrease in maximum heart rate and cardiac output; however, we do not have enough data to be positive.

"The factor limiting oxygen uptake remains unsolved. Which is the limiting stage on the way from the mouth to the mitochondria? Is it on the regulatory side? Is some factor keeping the heart rate and/or blood pressure low even during maximum exercise? If the blood pressure could be increased to twice the normal level, it would certainly provide adequate perfusion of the active muscles. Apparently the baroreceptors are active during exercise. Hypoxia *per se* does not seem to influence cardiac output. If a large proportion of the body musculature is involved in maximum effort, and measurements are made of cardiac output, heart rate and stroke volume, and if hypoxic stress is now added by placing the subject in a low-pressure chamber at a simulated altitude of 14,000 feet, it does not influence the recorded cardiac output, stroke volume or heart rate; apparently, the heart does not know whether it is working at sea level or at simulated altitude. However, the maximum oxygen intake decreases in relation to the unsaturation of the arterial blood.

"I want to turn now to the biopsy results mentioned earlier. May I emphasize that we are examining mitochondria in healthy subjects. I think that, in the future, intracellular events will be studied increasingly, and many important findings will arise from such investigations. Our studies have shown that during prolonged exercise the glycogen depots are depleted. It was once claimed that carbohydrate was the only fuel available to skeletal muscles; however, several factors seem to influence muscle metabolism. The first is diet: if the subject is given a carbohydrate-rich diet, more carbohydrate will be oxidized; if he receives a fat diet, relatively more fat will be burnt. A second factor is the work load. Our data suggest that during work of light or moderate intensity, combustion is divided rather evenly between fat and carbohydrate; however, during severe exercise, glycogen is the preferred fuel, and if the glycogen depots are emptied the work load must be reduced. The interpretation of such findings is complicated by the fact that glucose is essential to the central nervous system; exhaustion may thus be experienced before the glycogen depots are actually emptied. Migrating birds, such as the humming bird, burn fat exclusively; this is a wise arrangement, because glycogen storage becomes involved with water storage. For each gram of glycogen that is stored, 2.7 grams of water are also stored. If the migrating bird filled its depots with glycogen, it would be too heavy for take-off. Fat is a compact and relatively light fuel. Some animals burn almost nothing but fat in their muscles; some burn almost nothing but carbohydrate, and some burn a mixture. On a carbohydrate-rich diet, the respiratory quotient is higher than on a fat diet, and thus in prolonged exercise the respiratory quotient will be reduced. Well-trained subjects have a capacity to work at a heavy work load, where the free fatty acids contribute relatively more than the carbohydrates to the total metabolism.

"I would like to comment on the use and abuse of training for the untrained. Studies on the relationship between physical activity and cardiovascular health are very difficult, and I am afraid it may take a long time to get final proof. It is a critical question whether we should wait perhaps 100 years for this final proof, or whether we should take a chance that exercise may be beneficial. I accept indirect evidence and use a teleological approach. We are constructed for activity; once we had to run in order to survive. In those studies which have been reported, the risk of a coronary heart attack is two to three times higher for the inactive than for the active; the chance of surviving the first heart attack is also two or three times better in those who are active.

"Industrial activity can influence aerobic capacity. In my wife's material, there is a definite difference in the predicted maximum Vo_2 between different occupations; forest workers have a high capacity, followed by rock blasters, labourers, and carpenters; drivers and white-collar workers have poor values. However, as Professor Montoye has pointed out, in North America it may be difficult to find active people, because most of them have a set and inactive routine--the car to the office, the elevator up to their floor and down again, the car home, dinner, television and bed; there are some who claim to be active--they played golf two or three weeks ago and plan to play golf within another two weeks!

"In order to maintain a good functional capacity, some exercise is essential. If you want to move from point A to point B, it doesn't matter whether you walk slowly or run quickly, the energy required is about the same. This is important when discussing obesity. The doctor may say to his obese patient, "I prescribe that you walk five miles per hour, 30 minutes per day and after one month you will lose 1 1/2 pounds, everything being constant except for this walk," but the patient will not be impressed. However, if he claims that you should do this exercise every day for 10 years, and you will then lose 180 pounds, it is more impressive! You start to gain weight the day you get your first car, but it is only after some years you realize that your body weight has increased. To match this tendency, more calories must be used, and this extra usage will only be effective if continued over the years.

"In regard to whether fewer calories are consumed on the day of exercise, there are studies showing that as body temperature increases the appetite is depressed; this might explain these findings. In Sweden at least, the intake of iron, vitamins and some amino acids is proportional to caloric intake. When three or four thousand calories a day were consumed, the intake of these various items was adequate. Nowadays, most of the population is sedentary, and the daily energy expenditure of 50- to 60-year-old women is as low as 1500 Kcal. They thus ran a definite risk of getting too little iron, vitamins and amino acids. The physiological way to improve their nutritional status would be to increase their energy expenditure; more food would then be eaten and their nutrition would be correspondingly improved."

A list of 11 contributors from five countries have cooperated in a publication entitled: Biomedical Studies of the Musculo-Skeletal System. This includes eight original and previously unpublished papers, each written by a recognized authority and dealing with recent advances in the understanding of the functional significance of some part of the musculo-skeletal system. Techniques employed in research by some of the authors have been adapted from the fields of mechanics and engineering--techniques not commonly used in biological and medical investigations (46)

The USSR Ministry of Health conducted a 62-day study in which six healthy young men, ages 23 to 36, were confined to bed during this period. Movements without disturbance of the horizontal position were permitted to the subjects. Three of the men engaged in physical exercises daily according to a special program (47).

The investigation was regarded as having practical significance for the problems of selecting cosmonauts. The following conclusions were drawn from the study.

Two months of hypokinosis produced various disturbances in systems and organs. The extent of these disturbances and their pathogenesis differed from subject to subject. The most marked disturbances were in those subject systems which had some deficiency prior to the experiment

The translation of the above did not state whether or not the individual men were isolated. Nor was any information given concerning the initial medical and psychiatric status of the men reported. Diets fed before and during the bed rest also were not described, with no analysis of the calorie and nutrient content of the foods supplied. The type of exercise also was not described.

THE HUMAN SKELETON

The human adult skeleton contains about 206 bones, according to McLean and Urist, (48). It was for a long time regarded as a fixed structure which once formed, remained unchanged indefinitely. Today it is regarded as a dynamic entity which participates in and responds to the ebb and flow of life as do other body tissues. As living, developing, growing tissues, bone has a stake in the general metabolism which influences its function and is influenced by function. Some 25 to 30 per cent of bone is water. The remainder is a two-phase material of which 60 to 70 per cent is composed of mineral, which gives bone the ability to resist compression; the balance is made up of collagen (a protein) which provides its ability to resist tension. A bone can withstand many times the stresses to which it is subjected in the normal course of events.

If all of the organic material and water are removed, the remaining structure crumbles easily. On the other hand, if all organic salts are extracted from a long bone, the remaining structure, when fresh and moist, can be bent easily and tied into an overhand knot. After maturity, the proportions of fluid and of organic material may gradually decrease with age. Because of this and other factors, the bones of older persons often are easily broken, with healing becoming more difficult.

The organic portion of bones can be divided into: (a) cells, which constitute only a minute fraction of the total weight of bone; (b) a fibrous matrix, formed largely of fibrils of collagen, which can be extracted as glue or gelatin, and (c) an amorphous (formless) ground substance, consisting largely of mucopolysaccharides (protein-sugar compounds). The ground substance may be regarded as condensed tissue fluid, and along with the tissue fluid it is interspersed among the collagenous fibers. The organic matter is impregnated with the inorganic bone salts.

According to Hendricks and Hill (49), analyses show that the principal constituents of the mineral portion of bone are calcium, phosphate, and carbonate, with minor amounts of magnesium and sodium. Bone gives an X-ray diffraction pattern similar to that of the mineral apatite, the unit of structure of which contains $Ca_{10}(PO_4)_6F_2$. Various substitutions, such as $(OH)^-$ for F^-, and Mg^{+2} for Ca^{+2} are known to occur in the apatite lattice. Although formulas for the calcium phosphate component of bone have been proposed since Berzelius (1945), it currently is widely accepted that sodium and carbonate are essential constituents of bone mineral.

Bone tissue is permeated with blood vessels, lymph channels, and nerve branches. The microscopic Haversian system is the structural basis of compact bone. This consists of cylindrical tunnels about 0.05 mm. in diameter called the Haversian canals which are aligned with the long axis of the bone. These branch irregularly, and contain small blood and lymph vessels and nerve fibers. Bone tissue is deposited around each Haversian canal in very thin cylindrical concentric layers known as lamellae. Many small cavities are found between the lamellae, called lacunae. Each of the lacunae contains an osteocyte, or bone cell. Each lacuna appears shaped like a cigar made irregular by the minute channels, called canaliculi, which exit from it, connecting it with other lacunae and with the Haversian canals. These cavities are filled with tissue fluids.

Bones may be classified in a number of ways. There are long bones, such as the humerous and tibia, which are found in the limbs. Short bones are somewhat cubical and are represented only by the torsal and carpal bones. The sternum, ribs, ilium, scapula, and some of the skull bones are known as flat bones and have outer layers of compact bone filled inside with spongy bone and marrow. These are designed to serve as extensive flat areas for the attachment of muscles and ligaments and to enclose body cavities, except for the scapula. Usually flat bones are curved and thick where tendons and fascia attach and thin, nearly translucent, where the fleshy muscle fibers attach directly to the bone. Finally, irregular bones, like the ischium, pubis, maxilla, and vertebrae, are adapted to special purposes.

The long bones are adapted for weight-bearing and for sweeping, speedy movements. They serve these functions well because of their tubular form, their broad and specialized articular surfaces and shapes at their ends and their great length.

The long tubular part of a long bone is called the shaft or diaphysis. It contains the hollow medullary cavity, filled with marrow and surrounded with compact bone. After maturity, the compact bone of the shaft blends gradually into the compact bone of the two ends. The proximal end is usually called the head. Both the proximal and distal ends typically display protrusions called condyles or tuberosities, which serve as attachments or pulleys for tendons and ligaments. The shapes of the articular surfaces commonly are specialized to enable the bone to fit securely into the conformations of its neighbor, and to determine or limit the kind of action possible at the joint. Each articular surface has a cap of hyaline cartilage. This articular cartilage functions to increase the smoothness of fit, prevent excess wear, absorb shocks, and prevent dislocation of the joints.

Toward the ends of long bones, the medullary cavity gives way to spongy or cancellous bones within the external layers of compact bone. Spongy bone is as hard as compact bone, but is arranged in a complex grillwork. These bars of latticework are called cancelli or trabeculae. The basic tubular structure of long bones conserves weight, at the same time providing great resistance to stress and strain. The tensile strength of compact bone is 230 times greater than that for muscle of a similar cross-section.

The periosteum is a connective tissue which covers the outside of bones, except at the articular surfaces, where it is replaced by the articular hyaline cartilage. It has two layers, an outside layer of collagenous fibers, and a deep layer which is osteogenic (that is, capable of producing osteoblasts which may in turn develop into osteocytes). Periosteum is supplied with blood vessels and nerve branches. It is extremely sensitive to injury, and from it originates most of the pain of fractures, bone bruises, and "shin splints". Tiny processes, similar to small roots, penetrate the bone from the periosteum causing it to adhere to the outer surface of compact bone. Muscles are attached to periosteum, not directly to the bone.

The endosteum is a similar connective tissue which lines the medullary cavity and Haversian canals, and covers trabeculae of spongy bone. It, too, is osteogenic.

The primary function of red bone marrow is to manufacture red blood cells, although it also has osteogenic properties. At birth, red marrow fills both the medullary cavities and the intra-trabecular spaces of all long bones. As spongy bone increases in amount with age, the red marrow retreats to the intra-trabecular spaces, leaving the medullary cavity filled with fatty yellow marrow. Almost all the marrow in limb bones is yellow by the time of puberty, and red marrow normally is found only in parts of the ribs, skull, sternum, and vertebrae.

THE SCIENCE OF KINESIOLOGY

A recent book by Rasch and Burke on Kinesiology and Applied Anatomy (50) states the science of Kinesiology, or body movement, combines anatomy, physiology, and the science of body function and structure.

Aristotle is called the "Father of Kinesiology" because he was the first to describe the actions of the muscles and to subject them to mathematical analysis. Since Aristotle's day, many others have made contributions to the discourse on movement. A paper by Koch in 1917 on The Laws of Bone Architecture (50) constituted a classic contribution to the mathematical analysis of stress and strain in bone. This author concluded: "(a) bones are composed of compact and spongy material so as to produce maximum strength with minimum material; and (b) bones are designed in forms and structure to resist in the most economical way the normal maximum compressive stresses of body weight." He reasoned that muscle action was relatively unimportant in determination of bone structure and could be ignored.

Carey criticized Koch's theory of the unimportance of muscular tension in bone formation and asserted that powerful back pressure vectors due to muscular contration forces dominate the structure and growth of bones (50).

Space flight research problems have stimulated much additional research in this field. The development of the electronic stroboscope promises greater insight into musculoskeletal movements. This instrument is capable of exposures as short as one-millionth of a second and can record in a series of instantaneous photographs an entire sequence of movement. Advances in a related field--the science of aerodynamics--has greatly increased knowledge of movement of objects in space through investigations involving wind tunnels and other specialized research tools and artificial environments.

On earth the human organism has waged a constant war against the forces of gravity. Any movement by an individual involves the gravitational pull of the earth. This force can either be against or with the direction of the movement. In considering function and structure relationships, the factor of gravity is a major one. The amount of work which the heart muscle must perform at any time, regardless of the effort being exerted by other parts of the body, depends upon the relationship of the posture of the individual to the surface of the earth. If the person is lying down rather than standing up, less effort is required to pump the blood. In moving from a lying to a standing position, circulatory adjustments must be made and the gravitational pull on the venous return brings about adaptive mechanisms.

The phenomenon of stress has received a great deal of attention. The human organism attempts to adjust to any upset of the homeostatic equilibrium of the body in such a way as to restore its balance.

Elton (35), in his experience with army trainees, states that the femoral neck mav be involved in a stress reaction of such a character that it may constitute a major threat to the integrity of the lower extremity. The femoral shaft may also be involved in a stress reaction, with the anterior pelvic area less frequently affected. This orthopedic surgeon suggests that those who may be assigned to military training posts look for regional osteoporosis of a hip, or a foot, or a knee area, which later may manifest itself roentgenologically by a line of sclerosis at right angles to the cancellous trabeculation by a periosteal reaction, and at times by a frank bone fracture.

BONE STUDIES WITH EXPERIMENTAL ANIMALS

Bone density studies have not been confined to human subjects. Experimental animals have provided valuable data in this field. Geiser and Trueta (51) used 82 rabbits of both sexes from three to 12 months of age in five series of experiments, conducted for the purpose of studying bone rarefaction.

In the first experiment, involving 40 rabbits, partial incision of the calcaneal tendon was made. The animals were killed at short intervals in order to collect information on the successive changes taking place in the bones. The first changes which were visible in the bones on a radiograph, took place one week after tenotomy. The changes consisted of an early fading of the fan of the trabeculae of the calcaneum.

In the second experiment, 22 rabbits had their right hind leg immobilized by means of a padded plaster extending from the toes to just above the knee. The purpose of this experiment was to find the part which simple immobilization might play in causing bone atrophy. The immobilization in plaster-of-Paris caused changes similar to those obtained from tenotomy.

In Experiment 3, in which four animals were used, one leg of each animal was immobilized in plaster-of-Paris as in the previous experiment. In this case, a window was cut in the plaster over the calf muscles just large enough to admit a fine electrode through which faradic stimulation was applied twice daily in two animals and eight times daily in the other two animals. In the two animals stimulated twice daily, the effect did not differ markedly from the bone rarefaction shown in the animals in Experiment 2 who were immobilized without stimulation. In the two animals with more extensive stimulation, there was very slight bone rarefaction limited to the fan of trabeculae in the tuber calcanei. After four weeks, bone rarefaction had increased somewhat without reaching the severity of that observed in animals in the former experiment immobilized for the same period.

Severe trauma is considered as directly responsible for causing bone rarefaction by some investigators although these authors stated that they were not aware of any evidence to suggest that it is not the muscular inhibition after severe injuries affecting the bones, which causes bone resorption. As a result, another experiment was conducted. In Experiment 5, the authors used 14 rabbits to study the effect of fracture together with plaster immobilization. In this phase of the research, an injury in the form of an osteotomy was combined with immobilization. Following the application of the fracture, the bone fragments were stabilized by internal fixation with wire, after which the limbs were immobilized with plaster.

In this case, the same rarefaction was observed in the radiographs as when simple immobilization was imposed, although the bone rarefaction was somewhat more severe than after tenotomy. Collateral angiographs exhibited a marked increase in vascularization, both in Experiments 1 and 5.

In summarizing the study, the authors stated that they succeeded in causing rarefaction of the calcaneum of animals in five experiments soon after the bone was relieved from muscular compressing forces; new bone was generated when the calcaneum again was subjected to the stresses and strains of muscle contraction.

In the calcaneum of the rabbit, the authors concluded that the presence or absence of pressure forces is accountable for the balance between bone formation and bone removal.

In these experiments, bone rarefaction was found to be characterized by a great increase in the vascularity of the bone. This increase ceased when the bone reached its final low bone density. Thus, vascular over-activity accompanied the removal of bone, whereas bone reconstruction was accompanied by a more localized increase in vascularity.

Lipschütz and Audova of Estonia (52) conducted a study of the atrophy of muscle under different conditions, using the rabbit as the experimental animal. These investigators concluded that sectioning of the tendon Achilles in rabbits caused atrophy of muscle which was nearly as great as that caused by nerve section. The atrophy was caused by the great decrease in work, i.e. by inactivity, according to these investigators.

Karmosh and Saville (53) used rabbits in a study of the motor denervation on muscle and bone in the hind limb. Unilateral motor denervation of rabbit hind limbs was performed via hemilaminectomy. The muscle weight and bone ash weight on the paralyzed side was expressed as a fraction of that on the control side. Total radioactivity as well as specific activity ratios of bone ash were also determined.

Muscle and bone atrophy started immediately, continued for 10 weeks and then stopped. Bone accretion did not change until sometime between 10 days and four weeks when it increased by about 30 per cent.

The findings suggest that muscle atrophy leads to bone atrophy. The mechanism of the latter is by increased bone resorption followed by increased accretion until equilibrium is reached and the atrophy stops.

McArdle (54) studied the metabolic stress of endurance swimming in the laboratory rat. By means of closed circuit spirometry the oxygen consumption of 17 trained, male albino rats was determined under conditions of rest and swimming with no weight and with loads equivalent to 0.5, 1.0, 1.5, and 2.0 per cent of the body weight attached to the tail. Upon completion of the exercise, recovery metabolism was recorded for 25 minutes. It was concluded: (a) The exercise oxygen consumption of nonweighted animals averaged 2.7 times the resting level. In animals able to swim without apparent difficulty, this increased with the addition of weight to 3.5-fold at a load equivalent to 2.0 per cent body weight; (b) With the addition of weight many animals had difficulty swimming at the surface of the water. This difficulty was reflected in a decreased aerobic metabolism which actually was less than the average of the nonweighted trials; (c) The oxygen consumption in milliliters per kilogram per minute at rest and at all levels of exercise was negatively correlated with body weight; (d) The rate of recovery was dependent upon the preceding exercise stress; i.e., the heavier the weight attached during swimming, the slower the recovery.

Williams, Mason, and McDonald (55) conducted a study on the rat in which bone density measurements were made throughout the life cycle of this experimental animal. These authors concluded that a low bone denisty index was accompanied by retarded growth and poor lactation. A high bone density level, on the other hand, signifies an adequate calcium status. The authors state, however, that the findings could not serve as a basis for establishing calcium requirements in human beings.

Williams et al. (56) studied the influence of mineral intake on bone density in humans and in rats. They found that bone density levels of young human adults showed variations which appeared to indicate the calcium status of the individual. Mature rats showed no significant changes in bone density, although a small and significant difference in calcium content with increasing intakes ranging from 0.1 to 0.5 per cent of calcium.

Young growing rats showed significant increases both in bone density and body calcium with increased intake ranging from 0.1 to 0.5 per cent.

Long term isolation stress in rats was studied by Hatch, Balazs, Wiberg, and Grice (57). These investigators found that rats isolated for long periods became nervous and aggressive, with caudal dermatitis (scaly tail). After 13 weeks of isolation, rats had heavier adrenals and thyroid and lighter spleen and thymus compared with rats kept in community cages. This indicates an endocrinopathy with hyperfunction of the adrenal cortex.

Slack studied the metabolism of collagen in an investigation of the metabolism of limb atrophy in the rat. For purposes of the study, massive tissue atrophy of one hind limb was produced in rats by a technique of denervation and dislocation of the hip, after which labelled glycine was administered at varying periods of time. Under the conditions of the study, the metabolism of muscle protein appeared to continue normally for at least 14 days after the injection of the isotope and 14 weeks after the operation (58).

Mueller et al. (59) investigated the calcium metabolism and skeletal dynamics of laying pullets.

Twenty-four pullets were fed a constant specific activity ration for 26 days, after which an identical but Ca^{45}-free ration was fed for 29 days. The specific activity of the egg shell calcium was used to determine calcium balance and parameters of skeletal metabolism. Of the calcium intake 78 per cent was absorbed, 8 per cent was excreted as endogenous calcium and 70 per cent was retained. Shell formation caused a significant increase in calcium retention and a shift of calcium from apposition in bone and endogenous excretion to mobilization from bone and shell deposition. From 4.3 to 4.9 g of the skeletal calcium participated in egg shell formation, of which 1 g was turned over daily. The size of the exchangeable bone calcium pool was related to the quantity of shell produced and was larger in pullets with a negative calcium balance than in pullets with a positive balance. The quantity of calcium removed from the skeleton per day was positively correlated with the size of the exchangeable calcium pool, the quantity of calcium absorbed per day and shell mass. The correlation coefficient between the specific activity of medullary bone and egg shell calcium was 0.845, confirming the important role of medullary bone in shell formation.

Crandall (60) conducted an experiment on dogs, in which he studied the effect of physical exercise on gastric secretion. He found that after vigorous exercise on a treadmill, there was a rise of 2^{o} + 4^{o} degrees F. in rectal temperature. In the last two or three hours of the digestive period following exercise, certain of the dogs showed an increase in excretory rate to a point well above normal. He concluded that his experiment confirmed the common belief that heavy exercise before a meal interfered with digestion, undoubtedly causing a redistribution of blood, causing more to be shunted to the muscles and less to the viscera.

Mack et al. (61) studied the physiologic and metabolic changes in Macaca nemistrina (pigtail monkeys) on two types of diets during restraint and non-restraint with emphasis on bone density changes. In this investigation four groups of Macaca nemestrina were fed two diets which differed in content of major nutrients, with Diet A higher in protein and Diet B higher in fat, carbohydrate and major minerals. Animals on each diet were held in restraint on couches for 35 days, with exposure to a Biosatellite simulated reentry profile involving centrifugation at 12 G on the day that the restraint period ended.

The right knee joints were immobilized by means of a wire extending from the mid-tibia into the proximal femur behind the knee joint, with the wire threaded in such a manner that it remained within the skinfold back of the knee. Twelve of the dogs either broke the wire or infection occurred, so that only 24 dogs completed the time-controlled series. Length of times of immobilization were: 1 1/2 weeks for six dogs, four weeks for three dogs, six to eight weeks for eight dogs, and nine to twelve weeks for seven dogs.

At the end of each period, the specified animals were sacrificed. Quadriceps and patella tendons were dissected. Tissues from the normal control limb of each dog were pooled with others from the same group. Experimentally treated limbs were prepared in the same way. Tissues were dried, defatted, ground, and stored dry until ready for use. Mucopolysaccharide extractions were made and aliquots were analyzed for hexosamines, uronic acid, sulfate and nitrogen. Unhydrolyzed mucopolysaccharides and hydrolyzed specimens were chromatographed for hexosamines.

The results showed that, after four weeks of immobilization, a significant loss of mucopolysaccharides had occurred and the loss persisted throughout the remaining periods of 8 and 12 weeks. At the end of 1 1/2 weeks, there was an increase in content of acid mucopolysaccharides attributed to wound-healing from the threaded wire. From the second week to the twelfth week there was a reduction in mucopolysaccharides of 20 to 40 per cent.

The period of restraint was followed by 35 days of reconditioning of the formerly restrained animals in cages, with the same diets continued. Two groups of unrestrained primates were placed on the respective diets and were kept in cages for 70 days of the experiment except for exposure to the reentry profile on the same day that the restrained primates were exposed. Bone mass was measured periodically in all primates by the method of radiographic bone densitometry, with 17 anatomic sites in the skeletal system evaluated. Bone density was improved significantly in most skeletal sites when the diet containing the higher levels of calcium and phosphorus was the sole experimental factor changed. Restraint had the opposite effect, with loss in skeletal mass found in both dietary groups when this factor was applied. The special diet had a greater effect on improving bone density during the reconditioning period which followed restraint than during the restraint period itself.

Gross et al. carried on an investigation of calcium, nitrogen, and phosphorus mobilization resulting from conditions of inactivity in Macaca irus monkeys. In this experiment, calcium, phosphorus, and nitrogen balance resulting from conditions of inactivity were studied. The monkeys were inactivated by three different methods: surgical denervation, plaster casts, and tranquilization. The monkeys were placed in the plaster casts either in the sitting position or in the supine position. Balance studies were conducted by measuring food intake and urine and fecal output of calcium, nitrogen, and phosphorus. Calcium was measured by flame photometry, and nitrogen and phosphorus were measured colorimetrically. The animals inactivated by surgical denervation and plaster casts experienced what appeared to be a negative nitrogen balance which could be attributed to disuse atrophy of skeletal muscles. The urinary phosphorus excretion increased in the inactivated animals by severalfold but there was no overall reduction in the total phosphorus excreted. No noticeable changes in calcium balance were observed in the inactivated animals (62).

Kaplan et al. (63) conducted an investigation of the evaluation of animals continuously exposed to a 5 psia pure oxygen space cabin atmosphere for eight months. The purpose of the study was to contribute to the selection of atmospheric gases for space capsules. In the experiment, 117 animals of four species were exposed continuously to a pure oxygen atmosphere at 5 psia total pressure for 235 days. Clinical observation, serial blood chemistries, biochemical determination of hepatic cellular respiration, and histopathologic examinations revealed no evidence of systemic oxygen toxicity. Light microscopy revealed changes in the lungs of dogs and electron microscopy revealed changes in the lungs of dogs and rats that could be related to the oxygen exposure. It is unclear whether these changes were the result of the prolonged exposure or of the sudden return to ambient air prior to sacrifice. They were not associated with any apparent pulmonary functional deficit.

Akeson and LaViolette (64) have pointed out that immobilization studies have neglected generally the consideration of connective tissue atrophy, dealing mainly with bone mineral loss. These authors reported on the connective tissue response to immobility in 36 dogs.

Chor and Dolkart (65) conducted an immobilization study with six young Macaca rhesus monkeys. Body and leg casts were applied in an attempt to reduce the activity of the gastrocnemius muscles, although it was realized that "stretch" was still possible as well as other tonic reflexes. The authors, after histological and chemical analyses of the affected muscles, came to the following conclusions:

> (a) Disuse atrophy is a distinct entity and is simple in character, as revealed by histologic findings.
>
> (b) Disuse atrophy consists primarily of a uniform reduction of the bulk of each muscle cell, especially of the sarcoplasm. It is not attended by any evidence of degeneration or attempts at regeneration. Irritability to electrical stimuli remains unaffected.
>
> (c) There is no alteration in the proportions of water and nitrogen content.
>
> (d) Simple disuse is not associated with any demonstrable changes in the anterior horn cells of origin of the respective nerve supply, and the atrophy of the peripheral musculature due to disuse does not result in such changes.

Harrison and Fraser (66), on the basis of experiments with the diets of rats, concluded that pure calcium deficiency produces osteoporosis, or bone atrophy in rats while vitamin D deficiency as well as calcium deficiency leads to osteomalacia, or thin bones with wide osteoid seams. The retention of a dose of strontium in the osteoporotic rats is greater than normal, and this indicates rapid bone formation. The immediate cause of the bone thinning must therefore be an increase in the rate of bone resorption, contrary to the classical concepts of osteoporosis.

Osteoporosis has been defined as "too little bone". The present experiments show, however, that osteoporosis may occur even when new bone formation is proceeding at a rate greater than normal.

The mechanism for increased bone resorption in calcium-deficient rats is probably a dual one, mediated through the action of vitamin D on the skeleton. Vitamin D is known to increase bone resorption, and in osteomalacia the serum calcium is raised by this means, allowing calcification of the excessive osteoid matrix. In the osteoporosis produced in rats by pure calcium deficiency, the combined action of vitamin D and parathyroid hormone mobilizes the calcium stores already present in the animal's skeleton, in order to provide calcium for regions of rapid skeletal growth. Thus the skeleton shares its calcium store throughout a progressively larger mass of bone during growth, and becomes progressively thinner. The increased parathyroid activity leads to the rapid rate of bone formation. Here, again, there is presumably increased resorption of bone due to increased parathyroid activity, but in the absence of vitamin D the parathyroid hormone may not be able to exert its maximal effect, so that the blood levels of calcium are not raised sufficiently to allow calcification of the osteoid seams. It is well known that secondary hyperparathyroidism may accompany osteomalacia, both in animals and man.

PLAN OF PROCEDURE

This study is a component part of an extensive research program conducted in the Nelda Childers Stark Laboratory for Human Nutrition Research of the Texas Woman's University Research Institute, sponsored by the National Aeronautics and Space Administration. As noted in the INTRODUCTION of this Dissertation, the major purpose of this investigation has been to accumulate useful data on the value of programmed isometric and isotonic exercise on the maintenance of the integrity of skeletal mineral and related metabolic factors.

SUBJECTS OF THE STUDY

Cooperating in these studies were six healthy young adult male university students. These young men were carefully selected after passing extensive physical and psychological examinations. The following table gives the pertinent data on the subjects' heights, and then weight at the beginning and end of the study:

Subject	Age	Weight (pounds) Initial	Weight (pounds) Final	Height (inches)
AA	25	152.50	147.00	70.25
BB	22	152.00	149.50	72.00
EE	22	181.00	176.25	74.50
FF	20	155.00	149.00	69.50
GG	24	170.00	164.75	70.25
HH	21	170.00	165.50	69.00

GENERAL PROCEDURE USED IN THE STUDY

Throughout the entire study, the subjects were housed and fed in the metabolic ward of the Nelda Childers Stark Laboratory for Human Nutrition Research at the Texas Woman's University Research Institute. Specially trained dietitians planned and supervised the preparation of the meals which were optimum in all major nutrients. The daily food intake of each subject was recorded throughout the study by individual foods.

This study was conducted under close medical supervision. A record was made of height and weight changes throughout the study. Male orderlies attended to the hygienic needs of the subjects during immobilization, and safeguarded the movements of the subjects.

PERIODS OF THE STUDY

This study consisted of two bed rest periods accompanied by ambulatory periods as follows:

Equilibration Period, 29 days, June 3 - July 3, 1968

Bed Rest Number I, 28 days, July 3 - July 30, 1968

Interim Ambulatory Period, 14 days, July 31 - August 14, 1968

Bed Rest Number II, 28 days, August 14 - September 11, 1968

Post-Bed Rest Period, 14 days, September 11 - September 24, 1968

EQUILIBRATION PERIOD

This period lasted for 29 days. The six subjects led a normal life while engaged at various tasks in the laboratory, together with moderate exercise, for eight hours each day. They were required to be in bed at 10:30, with the lights out and the bed cubicles darkened from 11:00 P.M. until 7:00 A.M.

BED REST PERIOD NUMBER I

During this phase of the study, the subjects were immobilized for a period of 28 days. Throughout immobilization, the men assumed a horizontal position on a single bed equipped with one pillow. They were encouraged not to lift their heads, although very limited movement of the arms and legs was allowed. Reading was done with the aid of glasses equipped with prismatic lenses, and television was watched on hospital type television sets. During this period of immobilization, trained male orderlies were present around-the-clock to attend to the hygienic needs of the subjects and to safeguard their movement. Each subject was spoon fed by one of the three dietitians, with a record kept by weight and measure of their individual daily food consumption.

INTERIM AMBULATORY PERIOD

During this 14-day period of ambulation, the six subjects engaged in compulsory physical activity. They worked in the laboratory during the periods when they were not engaged in a physical exercise program. Meals were consumed in the metabolic ward under the supervision of the dietitians.

BED REST PERIOD NUMBER II

The variable in this 28-day bed rest was the introduction of an exercise program using the Exer-Genie and Exer-Grip Exercisers. Measurements of foot and hand action, squeeze, and isometrics were made with the Lufkin Anthropometric (woven) Tape with a Gurlick Spring Attachment (3176ME). The measurements of hand and foot action were started on the second day of immobilization, while the squeeze exercise was introduced on the third day and isometrics were begun on the fourth day of immobilization. Subjects AA, EE, and GG were allowed to exercise at will, while Subjects BB, FF, and HH exercised four times daily according to a firm schedule which was closely supervised.

Each desired exercise period called for by the "At Will" group also was supervised and conducted in the standard manner; but this group did not exercise unless they desired to do so.

POST-BED REST PERIOD

This final period of the study lasted for 14 days. During this time, the subjects engaged in supervised physical activity as much as their physical condition permitted. They also performed limited duties in the laboratory whenever they were not exercising. They re-entered the university at the beginning of the 1968-69 term.

DIETARY PLAN

Menus were planned so as to yield 2,600 calories per day, 90-100 grams of protein, 280 grams of carbohydrate, 120 grams of fat, 18-20 milligrams of iron, 10,000 I.U. of vitamin A equivalency, 400 I.U. of vitamin D, 100-150 milligrams of ascorbic acid, and minima of 1.6 milligrams of thiamine, 1.8 milligrams of riboflavin, and 21 milligrams of niacin. A minimum of 250 milligrams of magnesium was supplied daily. The meals were planned to fall in a four-day rotation cycle. All food items were analyzed chemically for calcium and phosphorus, with energy and other nutrients calculated from recently published tables.

During ambulation and bed rest, the food items were placed on individual dishes for the respective subjects after being recorded according to weight. Rejected food, which was minimal in amount, was weighed and placed in the day's record for the subject involved.

During the ambulatory periods, the subjects ate as a group at a table in the dining room kitchen of the metabolic ward under supervision. During a bed rest period they were spoon fed in a supine position in bed by one of the dietitians, as noted.

Because another member of the research team was carrying on a study to determine various aspects of calcium metabolism on the same subjects, ^{47}calcium was administered during the first day of each of the two bed rest periods in order to find how rapidly the radioactive calcium would reach the serum, and the urine. The administration of this entity, which was incorporated into the milk fed during the morning of the first day of each bed rest, was not thought to have any effect on the results of the study reported in this dissertation.

EXERCISE PROGRAM

During the second of the two 28-day bed rest periods, the exercise program was initiated. The girth of limbs (left and right both for arms and legs) and trunk measurements were accomplished by the use of a Lufkin Anthropometric Tape with a Gurlick Spring Attachment, as noted. Anatomical skin surfaces were marked, using a laundry pen, to insure subsequent measurements being made at the same sites. These measurements were repeated each five days during Bed Rest Number II.

Because the Exer-Genie was selected as an exercise device to place aboard the spacecrafts for the Apollo VII and the Apollo VIII missions, it was suggested by scientists at the Manned Spacecraft Center, National Aeronautics and Space Administration that this exercise instrument be used in this bed rest study in preparation for these missions. In addition, the Exer-Grip, a hand squeezing exercise device chosen to study the specific effect of exercise in causing the phalanges and wrist bones to resist reduction in bone density during recumbency, also was used.

By the use of volunteers from the work force of the Laboratories, a number of trials were made on the Exer-Genie to find desirable settings for the Exer-genie cylinder. In Summary A, data were obtained from the use of this exercise instrument with the cylinder set at 3.0 pounds. The results are given in terms of: (a) initial and final temperature readings of the cylinder of the instrument; (b) resting blood pressure and final blood pressure of the volunteer who functioned in this case (Subject A); and (c) resting pulse rate and final pulse rate of the subject undergoing the test.

This test was run for six minutes at 60 strokes per minute.

Summaries B and C give the same types of data with Exer-Genie cylinder settings of 6.0 and 8.0 pounds, respectively. Many other tests were made with different subjects, with settings of 3.0, 6.0, 8.0, 10.0, and 12.0 pounds. Summaries A, B, and C, which are representative, show the response of subjects with different initial levels of blood pressure and pulse rate to different cylinder settings of the exerciser.

The trials mentioned above showed that periods of rest were essential if the exercise program decided upon was to be successful. Summaries D and E show two of the many trials which were made in which the rest periods and isometric exercise were interspersed with the isotonic leg exercise.

Summary F outlines the Final Exercise Program which was selected for use in the investigation from the many trials which were run. The Summary shows that four brief rest periods were provided, that isometric exercise with the Exer-Genie strap pulled up to the chest, followed by body stretching was included at three points, that hand exercise using the Hand Gripper was inserted at two points, and that isotonic leg exercise was followed for two periods, for a longer time than for any other activity.

In carrying out this program, the cylinder of the Exer-Genie was set at 8 pounds, the Metronome used for keeping time for the leg exercise strokes was set at one beat per second, with the entire routine performed four times daily at 9:00 A.M., 11:00 A.M., 2:00 P.M., and 4:00 P.M. by those who exercised regularly. Those who exercised "at will" called for the exercise whenever they chose to follow it, but they were required to run through the entire routine under supervision each time they chose to function. Figure 1 shows one of the subjects using the Exer-Genie in an isotonic phase of the program when the feet were being exercised while he was in a supine position.

SUMMARY A

DATA ON TEMPERATURE OF CYLINDER ATTACHED TO THE EXERCISER (THE EXER-GENIE) WHEN IT WAS SET AT 3 POUNDS IN THE ROUTINE AS OUTLINED, TOGETHER WITH THE BLOOD PRESSURE AND HEART RATE OF VOLUNTEER SUBJECT A

1. Resting Blood Pressure of Subject 78/120
2. Resting Pulse Rate of Subject 70/minute
3. Initial Temperature of Cylinder . . . . 25° C - 77° F.

   After 6 minutes of exercise at one stroke per second, temperature of cylinder . . . . 130° C - 266° F.

   Continue with exercise for 4 additional minutes then:

4. Final Blood Pressure 150/65
5. Final Pulse Rate 116/minute
6. Final Temperature of Cylinder . . . . 140° C - 284° F.

SUMMARY B

DATA ON TEMPERATURE OF CYLINDER ATTACHED TO THE EXERCISER (THE EXER-GENIE) WHEN IT WAS SET AT 6 POUNDS IN THE ROUTINE AS OUTLINED, TOGETHER WITH THE BLOOD PRESSURE AND HEART RATE OF VOLUNTEER SUBJECT F

1. Resting Blood Pressure of Subject 120/78
2. Resting Pulse Rate of Subject 72/minute
3. Initial Temperature of Cylinder . . . . 25° C - 77° F.

   After 6 minutes of exercise at 60 strokes per minute, temperature of cylinder . . . . 95° C - 203° F.

   Continue with exercise for 4 additional minutes then:

4. Final Blood Pressure 135/65
5. Final Pulse Rate 126/minute
6. Final Temperature of Cylinder . . . . 115° C - 239° F.

S U M M A R Y   C

DATA ON TEMPERATURE OF CYLINDER ATTACHED TO THE EXERCISER (THE EXER-GENIE) WHEN IT WAS SET AT 8 POUNDS IN THE ROUTINE AS OUTLINED, TOGETHER WITH THE BLOOD PRESSURE AND HEART RATE OF VOLUNTEER SUBJECT E

1. Resting Blood Pressure of Subject 110/74
2. Resting Pulse Rate of Subject 70/minute
3. Initial Temperature of Cylinder . . . . 25^{o} C - 77^{o} F.

   After 6 minutes of exercise at 50 strokes per minute, temperature of cylinder . . . . 100^{o} C - 212^{o} F.

   Continue with exercise for 4 additional minutes then:

4. Final Blood Pressure 142/70
5. Final Pulse Rate 100/minute
6. Final Temperature of Cylinder . . . . 125^{o} C - 257^{o} F.

SUMMARY D

DATA ON TEMPERATURE OF CYLINDER ATTACHED TO THE EXERCISER (THE EXER-GENIE) WHEN IT WAS SET AT 6 POUNDS IN THE ROUTINE AS OUTLINED, TOGETHER WITH THE BLOOD PRESSURE AND HEART RATE OF VOLUNTEER SUBJECT H

1. Resting blood pressure of subject 120/82
2. Resting pulse rate of subject 74/minute
3. Initial temperature of EXER-GENIE cylinder . 25° C - 77° F.

TEN SECONDS OF ISOMETRIC EXERCISE
FOLLOWED BY SIX MINUTES OF LEG EXERCISE
AT ONE STROKE PER SECOND
(Setting of Exer-Genie pounds) 6 pounds resistance

4. Blood pressure of subject 160/70
5. Pulse rate of subject 118/minute
6. Cylinder temperature of EXER-GENIE . . . . . 125° C - 257° F.

TWO-MINUTE REST

7. Blood pressure of subject 115/70
8. Pulse rate of subject 80/minute
9. Cylinder temperature of EXER-GENIE . . . . . 85° C - 185° F.

TEN SECONDS OF ISOMETRIC EXERCISE (NO INTERMEDIATE REST PERIOD) FOLLOWED BY SIX MINUTES OF LEG EXERCISE, ONE STROKE PER SECOND

10. Blood pressure of subject 140/65
11. Pulse rate of subject 100/minute
12. Cylinder temperature of EXER-GENIE . . . . . 145° C - 293° F.

TEN SECONDS OF ISOMETRIC EXERCISE (NO INTERMEDIATE REST PERIOD) FOLLOWED BY SIX MINUTES OF LEG EXERCISE AT ONE STROKE PER SECOND

13. Blood pressure of subject 130/60
14. Pulse rate of subject 86/minute
15. Cylinder temperature of EXER-GENIE . . . . . 70° C - 158° F.

SUMMARY E

DATA ON TEMPERATURE OF CYLINDER ATTACHED TO THE EXERCISER (THE EXER-GENIE) WHEN IT WAS SET AT 6 POUNDS IN THE ROUTINE AS OUTLINED, TOGETHER WITH THE BLOOD PRESSURE AND HEART RATE OF VOLUNTEER SUBJECT G

1.	Resting blood pressure of subject	120/80
2.	Resting pulse rate of subject	45/minute
3.	Initial temperature of EXER-GENIE cylinder .	25° C - 77° F.

TEN SECONDS OF ISOMETRIC EXERCISE
FOLLOWED BY SIX MINUTES OF LEG EXERCISE
AT ONE STROKE PER SECOND
(Setting of Exer-genie pounds) - 6 pounds of resistance

4.	Blood pressure of subject	130/65
5.	Pulse rate of subject	72/minute
6.	Cylinder temperature of EXER-GENIE	110° C - 230° F.

TWO-MINUTE REST

7.	Blood pressure of subject	120/55
8.	Pulse rate of subject	51/minute
9.	Cylinder temperature of EXER-GENIE	70° C - 158° F.

TEN SECONDS OF ISOMETRIC EXERCISE (NO INTERMEDIATE REST PERIOD) FOLLOWED BY SIX MINUTES OF LEG EXERCISE, ONE STROKE PER SECOND

10.	Blood pressure of subject	130/62
11.	Pulse rate of subject	61/minute
12.	Cylinder temperature of EXER-GENIE	95° C - 203° F.

TEN SECONDS OF ISOMETRIC EXERCISE (NO INTERMEDIATE REST PERIOD) FOLLOWED BY SIX MINUTES OF LEG EXERCISE AT ONE STROKE PER SECOND

13.	Blood pressure of subject	125/65
14.	Pulse rate of subject	60/minute
15.	Cylinder temperature of EXER-GENIE	95° C - 203° F.

SUMMARY F

EXERCISE PROGRAM FOLLOWED IN BED REST II OF THIS INVESTIGATION

Instruments employed: Exer-Genie and Hand Gripper;

Setting of Exer-Genie during bed rest, 8 pounds;

Position of bed rest subjects throughout the study, including the exercise period: subjects in horizontal position, completely recumbent and lying on the back;

Steps in the Exercise Program:

		Time
1.	Isometric exercise with strap of Exer-Genie pulled up to the chest, with subject stretching	10 seconds
2.	Leg exercise (Exer-Genie)	6 minutes
3.	Rest	2 minutes
4.	Hand - Fingers squeezing gripper	1 minute
5.	Rest	2 minutes
6.	Isometric exercise (Exer-Genie)	10 seconds
7.	Arm exercise (Exer-Genie)	6 minutes
8.	Rest	2 minutes
9.	Hand - Fingers squeezing gripper	1 minute
10.	Rest	2 minutes
11.	Isometric exercise (Exer-Genie)	10 seconds
12.	Leg exercise (Exer-Genie)	6 minutes
	Total isometric exercise	30 seconds
	Total isotonic exercise	20 minutes

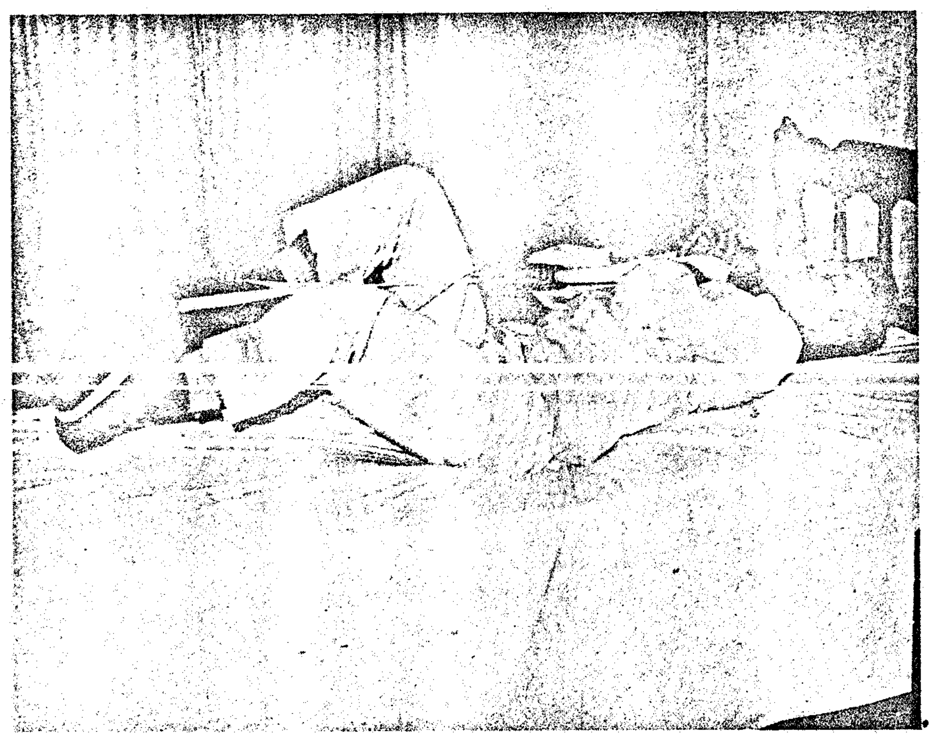


Figure 1. METHOD OF USING THE EXER-GENIE

IN ISOTONIC EXERCISE INVOLVING THE FEET

COLLATERAL CHEMICAL TESTS

Chemical tests were made on food, blood, urine and feces on the subjects of the study by members of the laboratory team. Each separate item of food was analyzed for calcium and phosphorus throughout, with the author of this report responsible in part for this contribution. Blood was analyzed once weekly by laboratory staff members as a measure of nutrition status. The analyses included in this part of the overall study included total protein, albumin, calcium, phosphorus, vitamin A equivalency, ascorbic acid, thiamine, riboflavin, and niacin.

In addition, analyses in a Beckman liquid scintillator were made by Marcia Clifton and her director, Dr. Elsa A. Dozier, for radio-active calcium fed in milk on the first day of each bed rest of this series, and non-radioactive calcium was analyzed by Dorothy Van Zandt and her directors, Dr. Ralph Pyke and Walter Gilchrist. The calcium excretions in urine and feces were compared in this report with the bone density changes during bed rest. Urinary analyses were made also for creatine, creatinine, 17-hydroxycorticosteroids, and hydroxyproline by other investigators who were working on the same subjects at the same time.

Chemical analyses on all components of the urine which were considered were made on pooled collections ending, respectively, at 8 A.M., 12 noon, and 8 P.M. Excellent circadian rhythms were derived from many of the urinary components for which analyses were made, especially calcium.

BONE DENSITOMETRY MEASUREMENTS

Methodology Employed. The method of measuring bone density in this study was the roentgenographic technique used at the Texas Woman's University, which had been developed by Mack and associates. The history of the method recently has been outlined in some detail by Mack (67).

The two earliest publications on this subject have been described by Mack and Smith (68) in 1938, and by Mack, Smith, Logan, O'Brien, and Bauman (69) in 1939. The earliest comprehensive reports in which this method of skeletal densitometry was applied to population groups were published by Lowther, Mack, et al. (70) in 1940, by Zavaz, Mack, et al. (71), also in 1940, by Mack, Smith, Logan, and O'Brien (72) in 1942, and by Mack and Urbach (73) also in 1942.

Introductions of step-by-step automation into the equipment have been described by Brown (74), by Mack (75) (76), by Mack, Brown, and Trapp (77), by Mack, Vose, and Nelson (78), by Nelson, Mack, and Vose (79), and by Vogt, Meharg, and Mack (80).

Descriptions of the bone density changes in the foot, hand, wrist, and distal radius and ulna during Gemini IV, V, and VII missions have been reported by Mack, Vose, Vogt, and LaChance (81) (82) (83), by Mack, LaChance, Vose, and Vogt (4), and by Mack and LaChance (1).

Descriptions of bone density changes during the missions of Apollo VII and VIII have been described by Mack, Vogt, Gilchrist, and Stover (5).

<u>Densitometer Assembly</u>. Figure 2 illustrates a section of the densitometer assembly employed at the Texas Woman's University in scanning the various sections of the bones x-rayed in this study. This portion of the assembly consists of an analogue computer system, the portions of which are outlined in the caption of this figure.

The sequence of operations needed to achieve calibration of a density curve and to integrate the area under the curve of a bone on the same radiograph using this assembly follows:

(a) The wedge roentgenographic image first is scanned for the purpose of providing the density calibration curve of that film on the first recorder. The scanning unit consists of an optical system, a plate stage, a drive mechanism for the plate stage, and a d-c amplifier for the recorder. The optical system includes a special tungsten lamp with plane optically polished windows, powered from a highly stabilized power pack having a constancy of output with 0.1 per cent for a-c line voltages between 100-125 volts at frequencies between 55 and 65 cycles. The beam from this lamp is focused on a photocell after passing through the x-ray plate being scanned. The film is mounted on the plate stage that is supported by ball-bearing rollers on a carriage rod, all accurately machined to very close tolerance.

A scale, mounted on the scanning unit and calibrated in millimeters, sub-divided by a vernier, indicates plate travel, and enables the operator to scan a number of precisely equal segments of the film trace. This scale also permits the operator to retrace exactly the same length of film on repeated scans, and serves as the guide for integrating the equal trace segments.

The plate travel is synchronized with recording chart travel to insure that quantitative measurements of density can be produced and reproduced accurately. A precision d-c amplifier with stabilized zero and stabilized gain multiplies the minute current from the photocell to a value measurable by a self-balancing potentiometer recorder.

(b) The first recorder consists of a Speedomax Type G self-balancing functioning Recorder having adjustable zero, adjustable span, and a full scale balancing speed of less than one second. This recorder indicates continuously the magnitude of the amplified photocell current and traces a graph on its chart in synchronism with the scanning unit plate travel.

A major feature of the complete assembly consists of a special d-c retransmitting slidewire mounted in this Speedomax Self-Balancing Potentiometer Recorder, with a moving contact on this slidewire driven in synchronism with the recording pen in an indicating pointer. This retransmitting slidewire is divided very precisely into 20 equal segments, each segment being shunted by an adjustable ten-turn potentiometer. An adjustable d-c voltage is impressed across the total slidewire; and the output of the slidewire is characterized by adjusting the potentiometer dials to provide a calibrated output from this slidewire from the trace of the reference wedge.

(c) The uncorrected wedge trace on the first recorder is scaled by the operator in the analogue system at 20 equal intervals, using a special transparent rule calibrated to provide direct setability of a calibration factor on the one thousand division dials of the 20 potentiometers.

Since the scale of the first recorder is determined by the per cent of transmitted light, and the calibrating retransmitting slidewire corrects the scale of the second recorder to standard wedge density, producing a straight line trace of the calibration wedge in conformity with the wedge slope, the x-ray absorption as indicated by its densitometer trace on the second recorder is related directly to the x-ray absorption of the standard wedge.

The second recorder receives, displays, and records the signal from the special calibrating retransmitting slidewire which serves as a function transformer, providing a line graph of the reference wedge and a calibrated density trace of the bone sample. Its graph also is synchronized with the automatic scanning.

Finally, a retransmitting potentiometer is driven from the output shaft of this second recorder and actuates an Instron electronic integrator which is located in the base of this unit. The Integrator provides a digital readout proportional to the area under the calibrated densitometer trace of the bone section which has been scanned on the second recorder.

The TWU bone densitometric system had the addition of a small digital computer to the analogue system about three years ago. The revised instrumentation is shown in Figure 3. This has been cited above (80).

The light transmission data from the film, in the form of a voltage functional with per cent light transmission, are measured with the same modified Knorr-Albers Microphotometer as described above, except for the fact that the vacuum tube DC amplifier originally used in the unit has been replaced with a solid-state amplifier and power supply to provide additional stability. The film is held in the motor-driven assembly that traverses a light beam as described previously. In the analogue system, analogue computer techniques employing adjustable function potentiometers are utilized to calibrate the light-transmission in terms of wedge-thickness for each film. Calibrated data from subsequent bone scans then were integrated by means of the electromechanical (Instron) integrator, as described. These systems required that several step-by-step adjustments and calibrations be performed by an operator.

In the new assembly, the output of the scanning unit is connected through an amplifier (Astrodata Model 885) to a small digital computer (a Digital Equipment Computation PDP-8 supplied by the Kaman Company). An analog-to-digital (A/D) converter formats the data for direct processing by the computer, and the computer samples the data at specific intervals of time. Programming and operating control is effected through a teletypewriter unit (TTY) that includes a paper-type input/output (I/O) feature.

The computer has been implemented to perform computations similar to the functions performed by the previous analog system. First, the wedge image is scanned and the resulting light transmission data are stored in terms of distance along (or thickness of) the wedge. Second, the bone image is scanned and the resulting light transmission data are stored. After both the wedge and bone scans have been completed, the computer calibrates the stored bone scan data in terms of equivalent wedge thickness by using the stored wedge scan data. The calibrated bone data then are integrated along the scan by using a trapezoidal approximation integration formula.

The present system offers an advantage over the earlier system in providing an automatic means for calibrating the film in terms of the light transmission of the aluminum alloy wedge, the roentgenogram of which is taken simultaneously with the roentgenogram of the bone of interest.

The previous system has been evaluated in extensive detail. The present technique also has been evaluated to show the reproducibility of successive scans. The results have been compared with the output of the analog system, with the finding that the analog and digital systems provide essentially the same results. The digital technique, however, offers a more rapid procedure for analyzing the films; and it reduces the technical error that could be introduced by the operator in measuring the heights of the uncorrected initial wedge trace at 20 points and adjusting the corresponding potentiometers. The analogue system is used at frequent intervals for comparative calibration purposes.

Exposure of Roentgenograms. In the study described in this report, roentgenograms were made of the experimental subjects approximately every other day during the ambulatory periods, and daily while the subjects were at bed rest, with very few exceptions. The foot and hand constituted the parts of the anatomy of which roentgenograms were made, as had been done in studies with the astronauts. The left foot was exposed in lateral and the left hand in posterior-anterior aspect.

Each exposure had a radiation level of 167±2 milliroentgens, a very minor amount.

Calibration of exposed films is effectuated by placing an aluminum alloy reference wedge on each film adjacent to the bone to be evaluated. The alloy in the wedge was selected because it exhibited an x-ray absorption coefficient similar to that of bone. The wedge serves as a means of correcting any bone scan which is traced by first correcting the trace of the wedge for deviations resulting from slight differences in film characteristics or development techniques, as has been described above. While being x-rayed, the subject is covered except for the part of the being x-rayed with lead impregnated vinyl sheeting. See Figures 4 and 5.

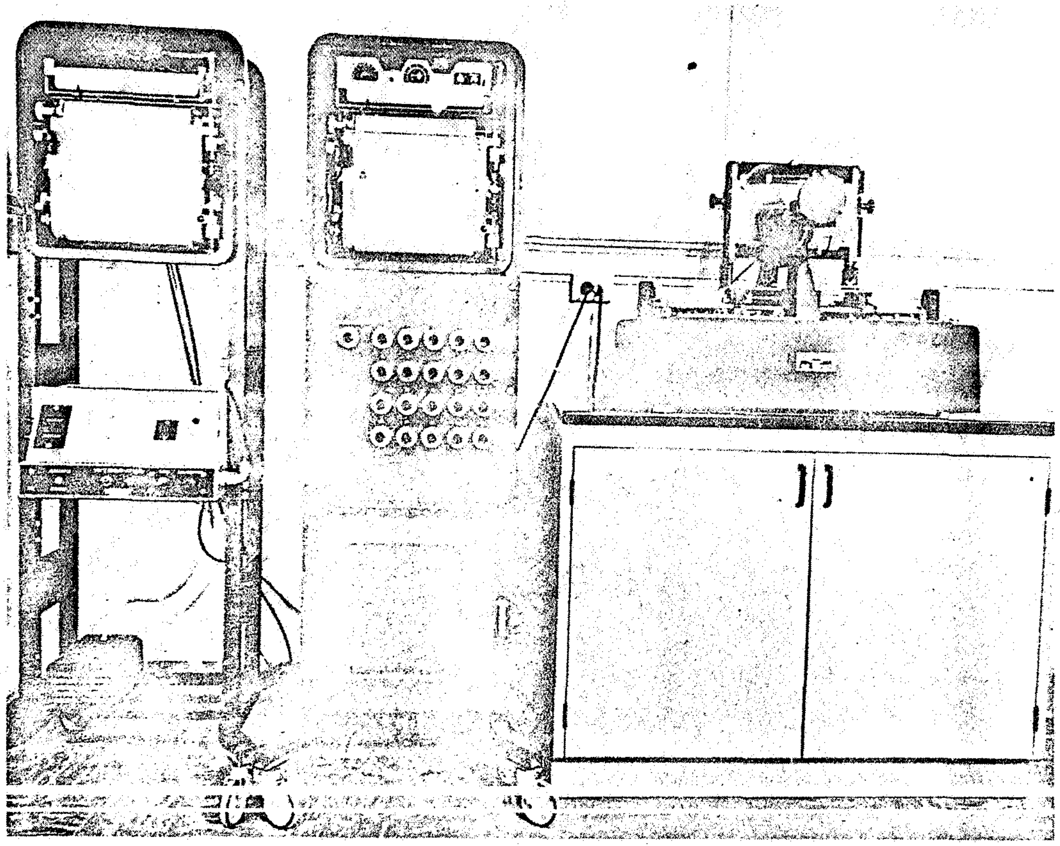


Figure 2. Analogue Computer Assembly. The equipment consists of five major sub-assemblies, all designed to operate together as a completely integrated system. The basic units of the overall assembly were the following.

(a) A modified Knorr-Albers scanning unit (unit at right of the figure);

(b) A Speedomax Model G transmitting recorder (unit in center of the figure);

(c) A series of 20 potentiometers in the same panel as (b);

(d) A Speedomax Model G recording potentiometer (unit at left of the figure), and

(e) An Instron Integrator (unit in the panel under Unit d).

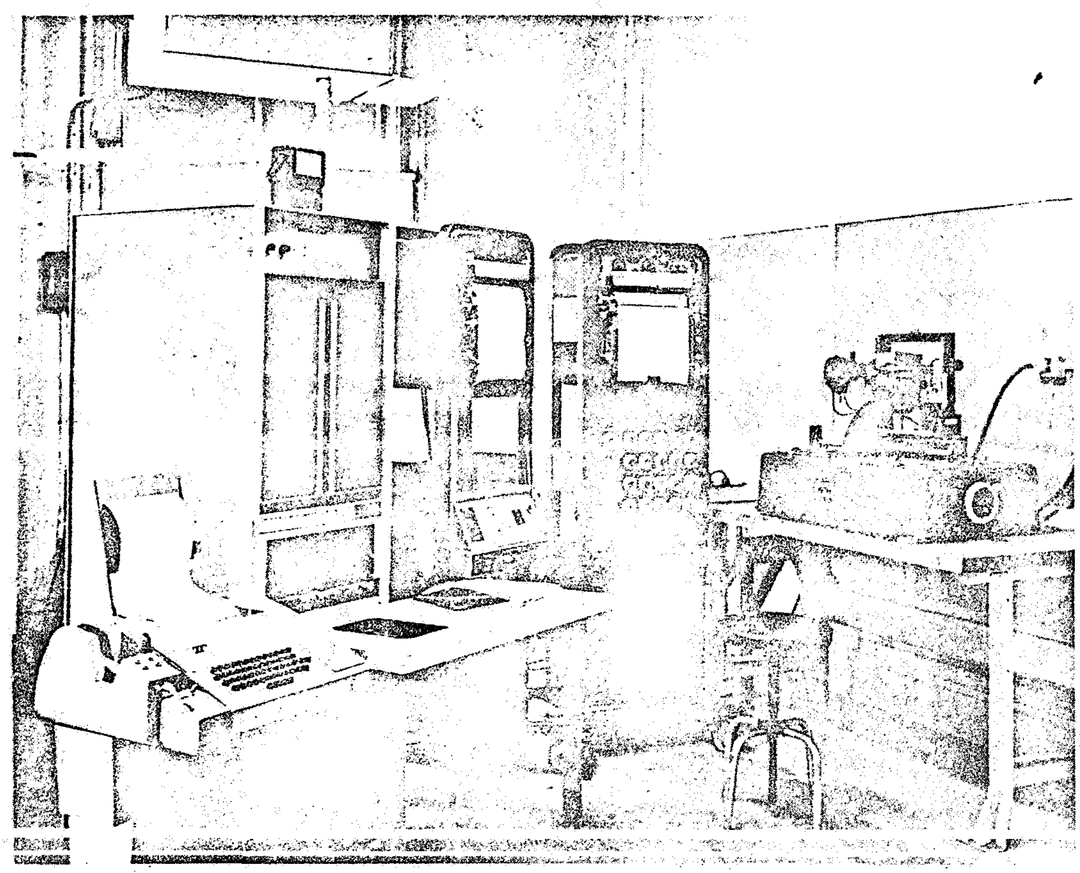


Figure 3. Digital-Analogue Computer Assembly. This is the bone densitometer assembly used in scanning sections of bone radiographs in order to evaluate their density in the Apollo VII tests. At the extreme right is the densitometer through which the calibration wedge is scanned by means of a standard light beam, after which the bone section to be measured is scanned. The two central sections are a part of the analogue computer assembly. At the extreme left is the digital computer, by means of which the test is programmed so that the density of the bone section is obtained without manual work on the two central recorders. The entire analogue assembly is used only periodically for calibration purposes.

Anatomic Sites Investigated. The anatomic sites in the foot at which bone sections are evaluated for skeletal density by means of the digital computer assembly which has been described include a single scan of the os calcis forming a section called "the conventional os calcis section" by those in the laboratory. In addition, parallel scans 1.0 millimeter from center to center of the scan are made above the central scan as far as there was no overlapping of other bones, and below to the bottom of this bone. These multiple scans cover approximately 60 per cent of the os calcis, or calcaneum. In addition, a central section of the talus is scanned.

Figure 6 shows the scans of the central sections of the os calcis and of the talus. Figure 7 shows a positive view of the os calcis with the multiple scans which completely cover this bone from the point in the proximal direction where overlapping of other bones begins to the bottom of the bone.

In the hand, crosswise multiple sections of hand phalanges 4-2 and 5-2 (Figure 8) cover these two bones entirely, with a single diagonal section evaluated across the capitate, and single scans across the distal ends of the radius and ulna. These sections give an indication of the skeletal density of the fingers, the wrist and the distal ends of two long bones in the arm.

Cancellous or trabecular tissue is represented in a major area of the os calcis and in the central portion of hand phalanx 4-2 and phalanx 5-2. Cortical or compact tissue is present in the perimeter of all of the individual bones, and is found in generous amounts in the distal end of the os calcis.

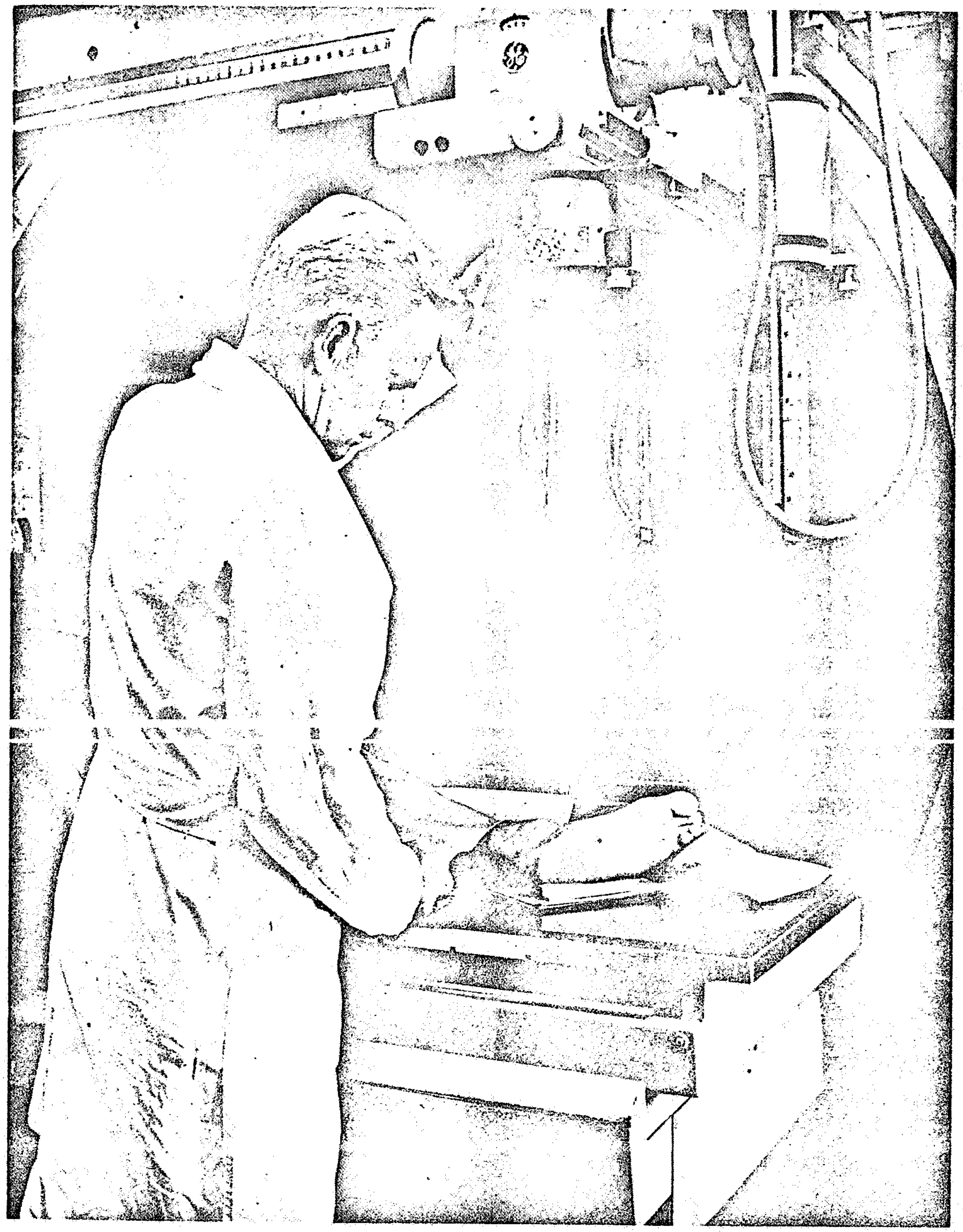


Figure 4. X-ray technologist positioning subject for exposing standard radiograph of os calcis

Figure 5. X-ray technologist positioning subject for exposing standard radiograph of hand

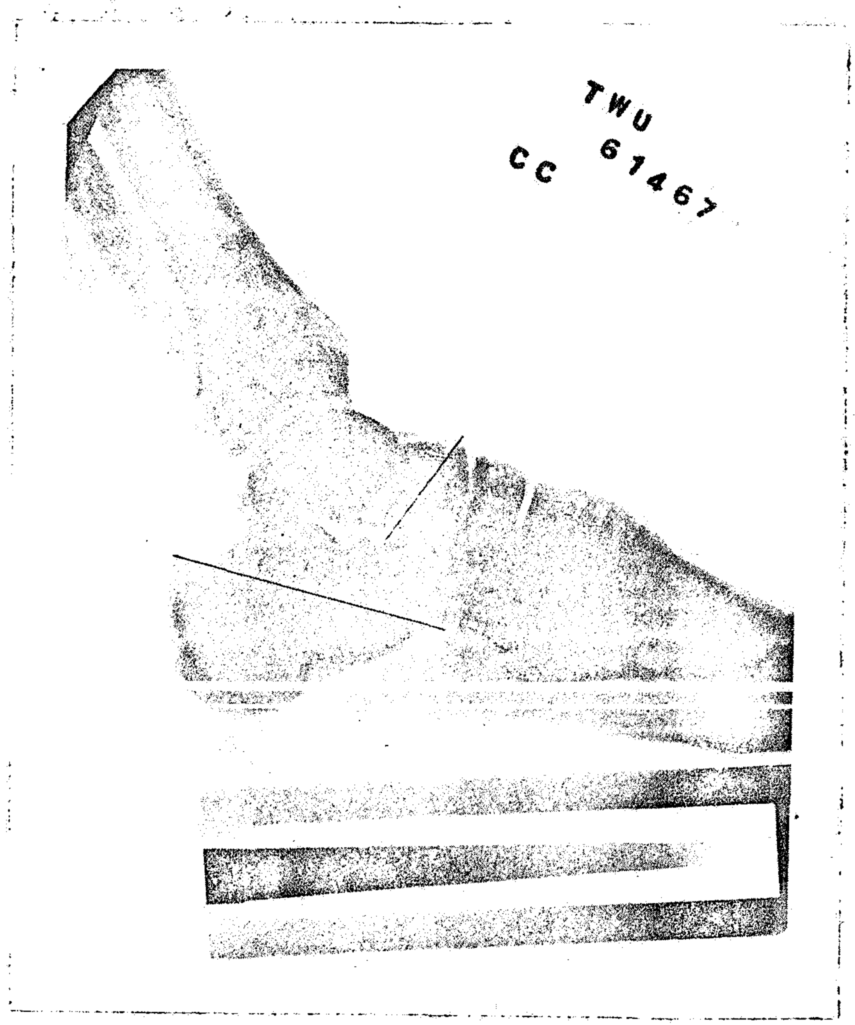


Figure 6. LATERAL ROENTGENOGRAM OF FOOT, INDICATING THE "CONVENTIONAL" SCANNING PATH OF THE CENTRAL OS CALCIS AND THE SCANNING SECTION OF THE CENTRAL TALUS

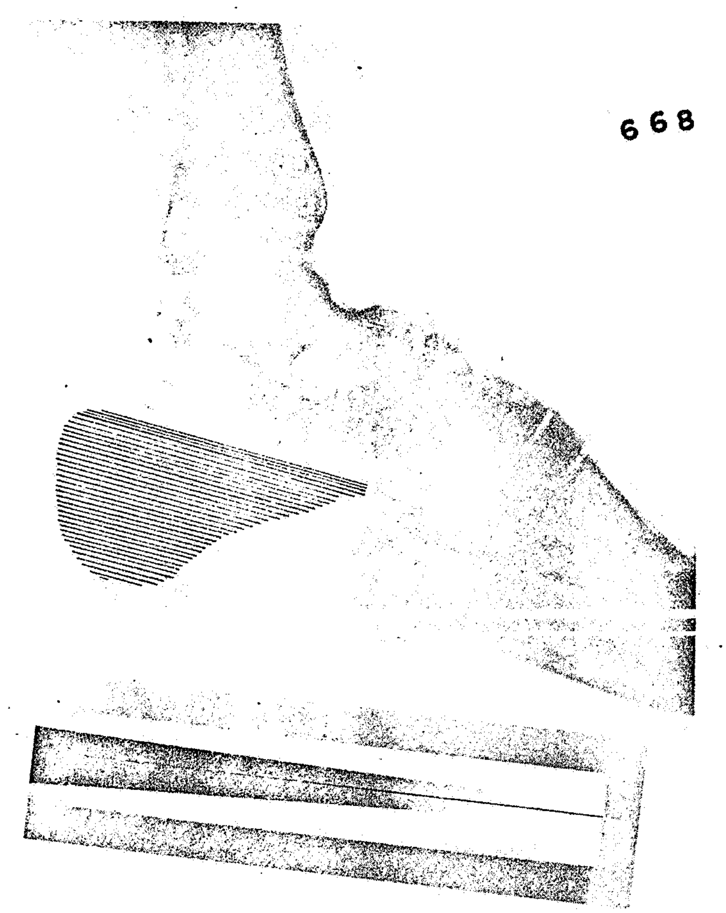


Figure 7. ROENTGENOGRAM OF FOOT IN LATERAL PROJECTION SHOWING THE ALIGNMENT OF PARALLEL SCANS COVERING APPROXIMATELY 60 PER CENT OF THE OS CALCIS

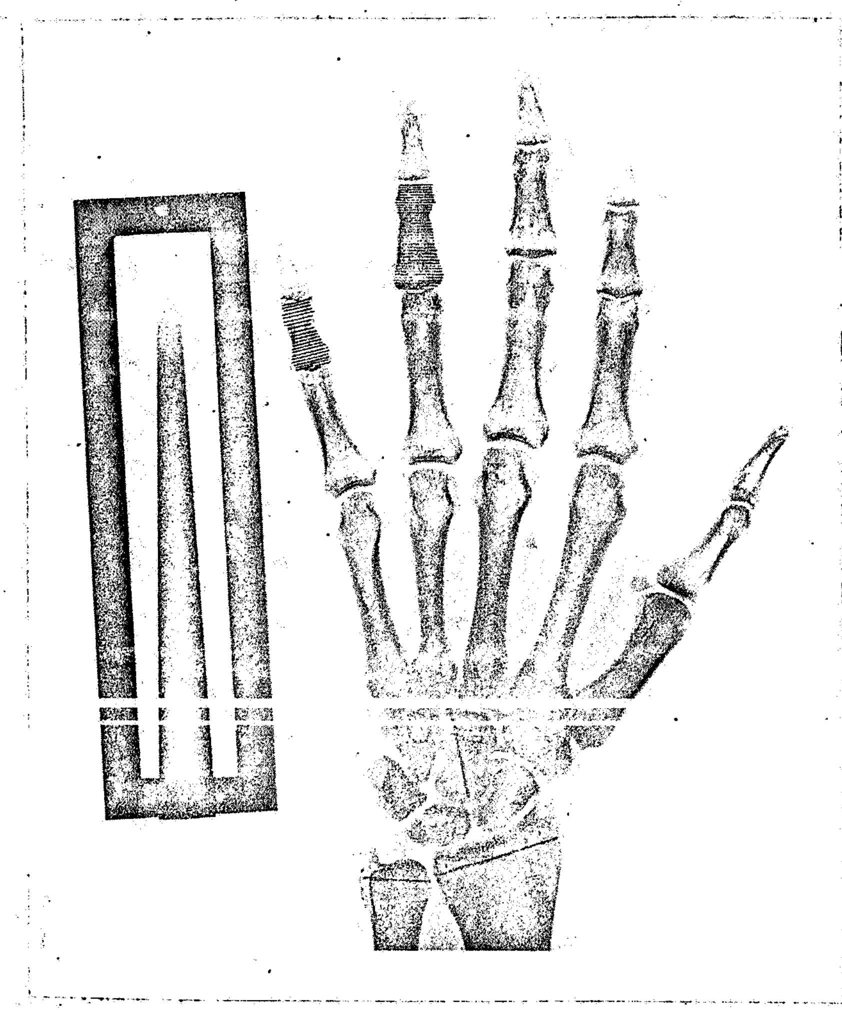


Figure 8. ROENTGENOGRAM OF THE HAND IN POSTEROANTERIOR PROJECTION, INDICATING PARALLEL SCANS WHICH COVER HAND PHALANGES 4-2 AND 5-2, AS WELL AS SINGLE SCANS FOR SECTIONS ACROSS THE DISTAL ENDS OF THE RADIUS AND OF THE ULNA

Standardization of Radiographs. Early in the period of work in this laboratory, a phantom was used to find whether or not randomly selected films in each new box purchased gave standard results, or whether possible manufacturing changes in processing may have resulted in differences in film spectral sensitivity or speed. This system has been continued. Kodak Type Industrial Film AA placed in cardboard holders is used for x-raying appendages. Prevention of variations in developmental technique have been studied extensively in these laboratories, with the finding that the concentrations of developing and fixing solutions change constantly with usage. Therefore, all films are hand developed in constant temperature equipment at 68±2°F., with one set of films, not to exceed 20 in number, developed at one time in new processing solutions, after which the solutions are discarded. The development and fixing times are regulated by stop clock.

Standardization of X-ray Machines. Because different x-ray units must be employed in some cases in some series of films, as for example in the Gemini astronaut series, the units must be calibrated and the conditions of exposure standardized. The method of calibration has been described in detail by Mack, LaChance, Vose, and Vogt (4).

Standardization of Radiographs by the National Bureau of Standards. Because "in house" standardization of radiographs has been regarded as important so that sequential films of one series produced in this laboratory may be compared with each other, work has been done cooperatively with the Data Corporation of Dayton, Ohio, to have unexposed and exposed x-ray films standardized by the National Bureau of Standards.

PRESENTATION OF DATA

TOTAL TIME OF EXERCISING BY EXPERIMENTAL SUBJECTS

As noted, the time consumed during the four daily periods set aside for exercising was recorded by stopwatch. The time consumed in minutes by the three subjects who exercised regularly (Subjects BB, FF, and HH) are given in Table I, Parts A, B, and C. The corresponding data for those who exercised "at will" appear in Table II, Parts A, B, and C.

Subject BB exercised for 2,194 minutes during the entire 28 days of Bed Rest II, when the exercise program was followed four times daily, except for the first two days when the time was consumed with blood and other special tests. Subject FF exercised for 2,181 minutes, and Subject HH for 1,913 minutes during Bed Rest II. The reason for the fact that Subject HH exercised during a lesser time than did the other two in this regular exercise category was that Subject GG, who began as a regular participant, decided after one week of the exercise period that he preferred to exercise "at will". Subject HH thereupon volunteered to exchange roles with Subject GG.

Of the subjects who exercised when they decided to do so, Subject AA exercised a total of 577 minutes during the days when exercise was followed. Subject EE exercised a total of 182 and Subject GG a total of 855 minutes. It should be noted, however, that the time expended in the exercise routine by Subject GG was expended to a considerable extent during the first week of this period, with many of the remaining days devoid of any exercise activity whatever.

TABLE I

DAILY EXERCISE SCHEDULE IN MINUTES OF SUBJECTS WHO EXERCISED REGULARLY THROUGHOUT THE STUDY

PART A. SUBJECT BB (EXERCISED REGULARLY)

Date	Amount of Exercise in Minutes				
	9:00 A. M.	11:00 A. M.	2:00 P. M.	4:00 P. M.	Total
August 16	-	-	4	-	4
17	-	12	12	16	40
18	20	20	23	23	86
19	23	23	23	23	92
20	23	23	23	23	92
21	23	17	23	23	86
22	23	23	23	23	92
23	23	23	23	23	92
24	23	23	23	23	92
25	23	23	23	23	92
26	23	23	23	23	92
27	23	23	23	23	92
28	23	23	23	28	92
29	23	23	23	23	92
30	23	23	23	23	92
31	23	23	23	23	92
September 1	23	23	23	23	92
2	23	23	23	23	92
3	23	23	23	23	92
4	23	23	23	23	92
5	23	23	23	23	92
6	23	23	23	23	92
7	23	23	23	23	92
8	23	23	23	23	92
9	23	23	23	23	92
10	23	23	-	-	46

TABLE I, CONTINUED

DAILY EXERCISE SCHEDULE IN MINUTES OF

SUBJECTS WHO EXERCISED REGULARLY

THROUGHOUT THE STUDY

PART B. SUBJECT FF (EXERCISED REGULARLY)

Date	Amount of Exercise in Minutes				
	9:00 A.M.	11:00 A.M.	2:00 P.M.	4:00 P.M.	Total
August 16	-	-	7	-	7
17	12	-	12	16	40
18	20	20	23	23	86
19	23	23	23	23	92
20	23	23	23	23	92
21	23	23	23	23	92
22	23	23	23	23	92
23	23	23	23	23	92
24	23	23	23	23	92
25	23	23	23	23 1/2	92 1/2
26	23	23	23	23	92
27	23	23	23	23	92
28	23	23	23	23	92
29	23	23	23	-	69
30	23	23	23	23	92
31	23	23	23	23	92
September 1	23	23	23	23	92
2	23	23	23	23	92
3	23	23	23	23	92
4	23	23	23	23	92
5	23	23	23	23	92
6	23	23	23	23	92
7	23	23	23	23	92
8	23	23	23	23	92
9	23	23	23	23	92
10	23	23	-	-	46

TABLE I, CONTINUED

DAILY EXERCISE SCHEDULE IN MINUTES OF

SUBJECTS WHO EXERCISED REGULARLY

THROUGHOUT THE STUDY

PART C. SUBJECT HH (EXERCISED REGULARLY)

Date	Amount of Exercise in Minutes				
	9:00 A.M.	11:00 A.M.	2:00 P.M.	4:00 P.M.	Total
August 16	-	-	4	-	4
17	-	12	12	16	40
18	-	-	17	23	40
19	-	-	23	23	46
20	-	23	23	-	46
21	-	-	23	-	23
22	-	-	23	23	46
23	-	-	23	23	46
24	23	23	29	29	104
25	23	23	23	23	92
26	23	23	23	23	92
27	23	23	23	23	92
28	23	23	23	23	92
29	23	23	23	23	92
30	23	23	23	23	92
31	23	23	23	23	92
September 1	23	23	23	23	92
2	23	23	23	23	92
3	23	23	23	23	92
4	23	23	23	23	92
5	23	23	23	23	92
6	23	23	23	23	92
7	23	23	23	23	92
8	23	23	23	23	92
9	23	23	23	23	92
10	23	23	-	-	46

TABLE II

DAILY EXERCISE SCHEDULE IN

MINUTES OF SUBJECTS WHO

EXERCISED "AT WILL"

PART A. SUBJECT AA (EXERCISED AT WILL)

Date	Amount of Exercise in Minutes				
	9:00 A. M.	11:00 A. M.	2:00 P. M.	4:00 P. M.	Total
August 16	-	-	4	-	4
17	-	12	12	16	40
18	-	-	8	-	8
19	-	-	23	-	23
20	-	-	23	-	23
21	-	-	23	-	23
22	-	-	23	-	23
23	23	-	-	-	23
24	-	-	-	23	23
25	-	-	-	-	-
26	-	-	14	-	14
27	-	-	-	-	-
28	-	-	-	-	-
29	-	-	-	-	-
30	-	-	-	-	-
31	-	-	-	22	22
September 1	-	23	-	23	46
2	-	23	-	-	23
3	-	23	-	23	46
4	-	23	-	23	46
5	-	23	-	29	52
6	-	23	-	23	46
7	23	-	-	-	23
8	-	-	-	-	-
9	-	23	23	-	46
10	-	23	-	-	23

TABLE II, CONTINUED

DAILY EXERCISE SCHEDULE IN MINUTES OF SUBJECTS WHO EXERCISED "AT WILL"

PART B. SUBJECT EE (EXERCISED AT WILL)

Date	Amount of Exercise in Minutes				
	9:00 A.M.	11:00 A.M.	2:00 P.M.	4:00 P.M.	Total
August 16	-	-	4	-	4
17	-	12	12	16	40
18	-	-	-	-	-
19	-	3	-	-	3
20	-	-	23	-	23
21	-	-	23	-	23
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-		-
25	-	-	-	-	-
26	-	-	-	-	-
27	-	-	-	-	-
28	-	-	-	-	-
29	-	-	-	-	-
30	23	-	-	-	23
31	-	-	-	-	-
September 1	-	-	-	-	-
2	-	-	-	-	-
3	23	-	-	-	23
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	20	20
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	23	23
10	-	-	-	-	-

TABLE II, CONTINUED

DAILY EXERCISE SCHEDULE IN

MINUTES OF SUBJECTS WHO

EXERCISED "AT WILL"

PART C. SUBJECT GG (EXERCISED AT WILL)

Date	Amount of Exercise in Minutes				
	9:00 A.M.	11:00 A.M.	2:00 P.M.	4:00 P.M.	Total
August 16	-	-	4	-	4
17	-	12	12	16	40
18	20	20	23	23	86
19	23	23	23	23	92
20	23	23	23	23	92
21	20	23	23	23	89
22	23	23	23	23	92
23	23	-	-	-	23
24	-	-	-	-	-
25	-	-	-	-	-
26	9	-	-	-	9
27	-	-	-	-	-
28	-	-	-	-	-
29	-	-	23	23	46
30	-	-	23	23	46
31	-	23	-	29	52
September 1	-	-	-	-	-
2	-	-	23	23	46
3	-	-	23	-	23
4	-	-	23	23	46
5	-	-	23	-	23
6	-	-	-	-	-
7	-	23	23	-	46
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-

CHANGES IN BONE DENSITY OF CENTRAL OS CALCIS SECTION

Table III gives the data for the central or "conventional" section of the os calcis whenever roentgenograms were made of the foot of the subjects who exercised regularly. The data are given in terms of Integrator Counts from the Densitometer Assembly, which could, if desired, be converted into calibration wedge equivalency or calcium hydroxyapatite equivalency. Since the conversion would not change the per cent change in the bone density values with time, however, no advantage was seen in not using the computer values directly.

Table IV includes the corresponding values for the central os calcis of the subjects who exercised "at will".

Comparisons of Bone Density Values of Central Os Calcis during Major Periods of the Study

Subjects Who Exercised Regularly. Of the regular exercise group, the subjects exhibited the following comparisons between the designated periods of the study, with respect to mean bone densities of the central section of the os calcis, in terms of Integrator Counts, and the probability of a significant difference between the means.

Subject BB	Means	Probability of Significance of Difference
Comparison of the Pre-Bed Rest . . .	12,262	$P<0.001$
Period with Bed Rest I	11,286	

	Means	Probability of Significance of Difference
Comparison of Pre-Bed Rest	12,262	N.S.*
With Interim Ambulatory Period . . .	12,041	
Comparison of Bed Rest I	11,286	P<0.001
With Bed Rest II	12,231	
Subject FF		
Comparison of Pre-Bed Rest	11,740	P<0.001
With Bed Rest I	10,579	
Comparison of Pre-Bed Rest	11,740	P<0.10
With Interim Ambulatory Period . . .	11,407	
Comparison of Bed Rest I	10,579	P<0.001
With Bed Rest II	11,456	
Subject HH		
Comparison of Pre-Bed Rest	13,395	P<0.001
Period with Bed Rest I	12,218	
Comparison of Pre-Bed Rest	13,395	N.S.
With Interim Ambulatory Period . . .	13,191	
Comparison of Bed Rest I	12,218	P<0.001
With Bed Rest II	12,944	

*N.S. means "no statistically significant difference".

	Means	Probability of Significance of Difference
<u>All Subjects who Exercised Regularly, with Data Pooled Together</u>		
Comparison of Pre-Bed Rest	12,466	P<0.001
With Bed Rest I	11,350	
Comparison of Pre-Bed Rest	12,466	N.S.
With Interim Ambulatory Period . . .	12,256	
Comparison of Bed Rest I	11,350	P<0.001
With Bed Rest II	12,220	

The data given in this section show that there was a high statistically significant difference between the Pre-Bed Rest and the Bed Rest I periods, with higher bone density values during the first Ambulatory Period than in the first horizontal recumbency period, when the subjects were not exercising. When the first Ambulatory Period, which preceded Bed Rest I, was compared with the Interim Ambulatory Period, which preceded Bed Rest II, there was no statistically significant difference in the data of all of the subjects pooled together, and in all but one of the individual subjects of the regular exercise group. In the case of the one exception, Subject FF, the difference was only slight, with significance shown only at the 10 per cent level of confidence. The results from this comparison of the two periods which preceded the respective bed rest periods show that there was little difference in the bone density of the anatomic site under consideration before the two respective bed rest periods began.

The statistical comparison of the central os calcis during Bed Rest I and Bed Rest II showed that, for those who exercised regularly, the bone density of this anatomic site was higher during the recumbency period when exercise was taken (Bed Rest II) than that during the period with no exercise (Bed Rest I).

Subjects who Exercised "At Will". Of the subjects who exercised only when they desired to do so, the following comparisons of the bone density of the central os calcis were seen for the designated periods of the study.

	Means	Probability of Significance of Difference
Subject GG		
Comparison of Pre-Bed Rest	10,443	$P<0.001$
With Bed Rest I	9,863	
Comparison of Pre-Bed Rest	10,443	N.S.
With Interim Ambulatory Period . . .	10,573	
Comparison of Bed Rest I	9,863	N.S.
With Bed Rest II	9,992	
Subject AA		
Comparison of Pre-Bed Rest	12,297	$P<0.001$
With Bed Rest I	11,694	
Comparison of Pre-Bed Rest	12,297	N.S.
With Interim Ambulatory Period . . .	12,284	

	Means	Probability of Significance of Difference
Comparison of Bed Rest I	11,694	P<0.05
With Bed Rest II	11,843	
Subject EE		
Comparison of Pre-Bed Rest	10,766	P<0.001
With Bed Rest I	9,851	
Comparison of Pre-Bed Rest	10,766	N.S.
With Interim Ambulatory Period . . .	10,666	
Comparison of Bed Rest I	9,851	N.S.
With Bed Rest II	9,902	
All Subjects who Exercized "At Will"		
Comparison of Pre-Bed Rest	11,169	P<0.001
With Bed Rest I	10,461	
Comparison of Pre-Bed Rest	11,169	N.S.
With Interim Ambulatory Period . . .	10,889	
Comparison of Bed Rest I	10,461	N.S.
With Bed Rest II	10,579	

Of the subjects who followed the exercise program only when they chose to do so, sometimes not even completing one entire unit of the program although they were being supervised for as long as they continued, the bone density of the central os calcis section during the Pre-Bed Rest Period surpassed that during Bed Rest I (no exercise) by differences for each individual subject which were highly significant ($P < 0.001$ in each case). With the data for all subjects pooled together for the same comparison, the same superiority was found, with the difference highly significant ($P < 0.001$).

In the case of these subjects, again there was no statistically significant difference between the central os calcis bone density values of the ambulatory period which preceded the first and that which preceded the second Bed Rest Period. When the two Bed Rest Periods were compared, the bone density values for the "at will" exercise subjects during Bed Rest II (with exercise) failed to surpass Bed Rest I (with no exercise) except in the case of Subject AA. In this case the Bed Rest with Exercise Period exceeded the Bed Rest Period with no exercise by a difference which was significant ($P < 0.05$). The level of probability of a significant difference, however, far less than was found for each subject who exercised regularly.

Subject GG, who exercised for a total length of time (855 minutes) which surpassed that of Subject AA (577 minutes), showed no statistically significant difference between the bone density levels of the central os calcis throughout Bed Rest II as compared with Bed Rest I. The reason for this finding undoubtedly emanated from the fact that the exercise time accomplished was grouped in the first few days of Bed Rest II, with far less time expended in exercising as Bed Rest II progressed, and with nine days devoid of any exercise whatever during this period.

With the data pooled for all of the subjects who did not exercise regularly, the Pre-Bed Rest ambulatory period markedly surpassed the period of Bed Rest I in bone density of the central os calcis section ($P<0.001$). The Initial and Interim ambulatory periods did not differ significantly in bone density of the anatomic site under discussion, nor was there any statistically significant difference between the results with respect to the mean of this bone section densities during the two bed rest periods.

Table III gives the complete data for the bone density values of the central os calcis section in terms of Integrator Counts and Calibration wedge equivalency for the subjects who had no exercise during Bed Rest I, but who exercised regularly during Bed Rest II. Table IV gives the corresponding data for those who did not exercise during Bed Rest I, but who exercised only when they desired to do so during Bed Rest II.

19	12,598.0	2.2676	11,541.0	2.0774	13,097.5	2.3576
20	12,171.[illegible]	2.1909	11,417.5	2.0552	[illegible]2,924.0	2.3263
21	12,263.0	2.2073	11,597.0	2.0875	12,845.5	2.3122
22	12,380.5	2.2285	11,572.0	2.0830	12,689.0	2.2840
23	12,397.5	2.2316	11,634.5	2.0942	12,558.0	2.2604
24	12,286.0	2.2115	11,551.0	2.0792	12,887.5	2.3198
25	12,195.5	2.1952	11,340.5	2.0413	12,879.0	2.3182
26	12,227.0	2.2009	11,567.0	2.0821	13,145.5	2.3662
27	12,309.0	2.2156	11,515.0	2.0727	13,136.0	2.3645
28	12,171.5	2.1909	11,444.5	2.0600	13,027.0	2.3449
29	X	X	X	X	X	X
30	12,288.5	2.2119	11,417.0	2.0551	13,069.0	2.3524
31	12,247.0	2.2045	11,680.5	2.1025	13,380.0	2.4084
September 1	12,269.5	2.2085	11,344.5	2.0420	12,988.5	2.3379
2	12,196.0	2.1953	11,387.0	2.0497	12,996.5	2.3394
3	12,313.5	2.2164	11,349.5	2.0429	12,985.0	2.3373
4	11,982.0	2.1568	11,318.5	2.0373	12,575.5	2.2636
5	12,002.0	2.1604	11,289.0	2.0320	13,394.0	2.4109
6	12,195.5	2.1952	11,165.0	2.0097	12,947.5	2.3306
7	12,244.0	2.2039	11,470.0	2.0646	12,856.0	2.3141
8	12,080.5	2.1745	11,390.0	2.0322	12,763.0	2.2973
9	12,304.0	2.2147	11,574.5	2.0834	12,995.0	2.3391
10	12,118.0	2.1812	11,630.5	2.0935	13,155.0	2.3679
Bed Rest II ended 2 p.m.						
11	11,928.0	2.1470	11,556.5	2.0802	12,533.5	2.2560
12	11,156.5	2.0082	11,555.5	2.0800	12,360.0	2.2248
13	11,356.0	2.0441	11,568.5	2.0823	12,892.0	2.3206
14	11,610.5	2.0899	12,095.5	2.1772	12,373.5	2.2272
15	X	X	X	X	X	X
16	11,499.5	2.0699	12,020.0	2.1636	12,857.0	2.3143
17	11,539.0	2.0770	11,979.5	2.1563	13,289.0	2.3920
18	11,469.0	2.0644	11,986.0	2.1575	13,305.0	2.3949
19	11,701.0	2.1062	12,038.5	2.1669	13,788.5	2.4819
20	11,766.0	2.1179	11,935.5	2.1484	13,097.0	2.3575
21	11,633.5	2.0940	11,863.0	2.1353	13,107.0	2.3593
22	X	X	X	X	X	X
23	11,602.5	2.0884	11,548.5	2.0967	13,708.0	2.4674
24	11,532.0	2.0758	11,027.0	1.9849	13,315.0	2.3967

13	11,359.5	2.0447	10,537.0	1.8967	12,337.5	2.2208
14	11,185.[illegible]	2.0133	10,528.5	1.8951	12,302.0	2.2144
15	X	X	X	X	X	X
16	11,186.0	2.0135	10,424.0	1.8763	12,475.0	2.2455
17	X	X	X	X	X	X
18	11,168.5	2.0103	10,415.5	1.8748	12,884.5	2.3192
19	11,128.0	2.0030	10,392.0	1.8706	12,895.5	2.3212
20	11,320.0	2.0376	10,373.0	1.8671	12,763.0	2.2973
21	11,288.5	2.0319	10,302.0	1.8544	11,856.5	2.1342
22	11,375.0	2.0475	10,293.0	1.8527	11,447.0	2.0605
23	11,183.0	2.0129	10,240.0	1.8432	11,565.5	2.0818
24	11,142.0	2.0056	10,277.5	1.8499	11,576.0	2.0837
25	11,139.0	2.0050	10,202.0	1.8364	11,678.0	2.1020
26	X	X	10,214.5	1.8386	X	X
27	11,285.5	2.0314	10,204.5	1.8368	11,656.0	2.0981
28	11,163.5	2.0094	10,156.0	1.8281	11,873.5	2.1372
29	11,243.5	2.0238	10,240.0	1.8432	11,438.0	2.0588
30	11,205.5	2.0170	10,124.0	1.8223	11,685.0	2.1033
Bed Rest I ended at 2 p.m. (No Exercise during this period)						
31	11,884.0	2.1391	10,284.0	1.8511	12,208.0	2.1974
August 1	X	X	X	X	12,820.0	2.2176
2	11,391.5	2.0505	11,342.0	2.0416	12,937.5	2.3288
3	X	X	11,577.5	2.0840	12,999.5	2.3399
4	X	X	X	X	X	X
5	11,985.0	2.1573	11,452.5	2.0614	13,250.0	2.3850
6	X	X	X	X	X	X
7	11,990.0	2.1582	11,469.5	2.0645	13,804.5	2.4848
8	11,828.0	2.1290	11,531.5	2.0757	13,785.0	2.4813
9	12,100.5	2.1781	11,689.0	2.1040	13,423.0	2.4161
10	X	X	X	X	X	X
11	X	X	X	X	X	X
12	12,662.0	2.2792	11,708.5	2.1075	13,096.0	2.3573
13	12,486.5	2.2476	11,605.5	2.0890	13,590.5	2.4463
Bed Rest II began (Exercise) 2 p.m.						
14	12,115.0	2.1807	11,256.0	2.0261	12,828.0	2.3090
15	12,263.5	2.2074	11,509.5	2.0717	12,808.0	2.3054
16	12,190.0	2.1942	X	X	12,804.0	2.3047
17	12,198.0	2.1956	11,491.0	2.0684	12,787.0	2.3017
18	12,234.5	2.2022	11,512.5	2.0722	12,958.5	2.3325
19	12,598.0	2.2676	11,541.0	2.0774	13,097.5	2.3576
20	12,171.5	2.1909	11,417.5	2.0552	12,924.0	[illegible]
21	12,263.0	2.2073	11,597.0	2.0875	12,845.5	[illegible]
22	12,380.5	2.2285	11,372.0	2.0930	[illegible]	[illegible]

7	11,650.5	2.0971	11,[illegible]	[illegible]		
8	X	X	X	X	X	X
9	X	X	X	X	X	X
10	12,222.5	2.2001	11,917.0	2.1451	13,216.5	2.3790
11	12,417.0	2.2351	11,801.0	2.1242	13,425.0	2.4165
12	X	X	X	X	X	X
13	12,334.5	2.2202	11,752.5	2.1155	13,306.0	2.3951
14	X	X	X	X	X	X
15	12,634.5	2.2742	11,675.0	2.1015	13,585.0	2.4453
16	X	X	X	X	X	X
17	12,542.5	2.2577	11,701.5	2.1063	13,341.0	2.4014
18	12,457.0	2.2423	11,783.5	2.1210	13,475.0	2.4255
19	X	X	X	X	X	X
20	12,394.0	2.2309	11,728.0	2.1110	13,010.5	2.3419
21	12,277.0	2.2099	11,801.5	2.1243	13,352.0	2.4034
22	X	X	X	X	X	X
23	X	X	X	X	X	X
24	12,588.0	2.2658	11,804.5	2.1248	13,300.5	2.3941
25	X	X	X	X	X	X
26	12,234.5	2.2022	X	X	13,410.0	2.4138
27	X	X	11,961.5	2.1531	X	X
28	12,328.0	2.2190	11,716.5	2.1090	13,093.0	2.3567
29	X	X	X	X	X	X
30	X	X	X	X	X	X
July 1	12,524.5	2.2544	12,577.5	2.2640	13,327.5	2.3990
Bed Rest I began, 2 p.m., no Exercise during the Bed Rest						
2	X	X	X	X	X	X
3	11,824.0	2.1283	11,491.0	2.0684	13,129.5	2.3633
4	11,716.0	2.1089	11,504.5	2.0708	12,526.5	2.2548
5	X	X	X	X	X	X
6	11,590.0	2.0862	11,614.0	2.0905	12,575.0	2.2635
7	11,462.0	2.0632	11,419.5	2.0555	12,436.5	2.2386
8	11,294.0	2.0329	11,381.5	2.0487	12,389.0	2.2300
9	X	X	X	X	X	X
10	X	X	X	X	X	X
11	10,954.0	1.9717	10,402.0	1.8724	12,652.5	2.2774
12	11,076.0	1.9937	10,585.5	1.9054	12,665.0	2.2797
13	11,359.5	2.0447	10,537.0	1.8967	12,337.5	2.2208
14	11,185.0	2.0133	10,528.5	1.8951	12,302.0	2.2144
15	X	X	X	X	X	X
16	11,186.0	2.0135	10,424.0	1.8763	12,475.0	2.2455
17	X	X	X	X	X	X
18	11,168.5	2.0103	10,415.5	1.8748	12,884.5	2.3192
[illegible]	[illegible]	[illegible]	10,392.0	1.8706	12,895.5	2.3212

TABLE III

BONE DENSITY VALUES OF CENTRAL SECTION OF OS CALCIS ON DESIGNATED DAYS OF THE STUDY

(Subjects who Exercised Regularly during Bed Rest II)

Date	Subject BB		Subject FF		Subject HH	
	Integrator Counts	A x 0.00018 Calibration Wedge Equivalency	Integrator Counts	A x 0.00018 Calibration Wedge Equivalency	Integrator Counts	A x 0.00018 Calibration Wedge Equivalency
June 3, 1968	11,864.5	2.1356	11,261.5	2.0271	13,851.5	2.4933
4	11,457.0	2.0623	11,252.0	2.0254	13,549.0	2.4388
5	X	X	X	X	X	X
6	X	X	X	X	X	X
7	11,650.5	2.0971	11,364.5	2.0456	13,682.0	2.4628
8	X	X	X	X	X	X
9	X	X	X	X	X	X
10	12,222.5	2.2001	11,917.0	2.1451	13,216.5	2.3790
11	12,417.0	2.2351	11,801.0	2.1242	13,425.0	2.4165
12	X	X	X	X	X	X
13	12,334.5	2.2202	11,752.5	2.1155	13,306.0	2.3951
14	X	X	X	X	X	X
15	12,634.5	2.2742	11,675.0	2.1015	13,585.0	2.4453
16	X	X	X	X	X	X
17	12,542.5	2.2577	11,701.5	2.1063	13,341.0	2.4014
18	12,457.0	2.2423	11,783.5	2.1210	13,475.0	2.4255
19	X	X	X	X	X	X
20	12,394.0	2.2309	11,728.0	2.1110	13,010.5	2.3419
21	12,277.0	2.2099	11,801.5	2.1243	13,352.0	2.4034
22	X	X	X	X	X	X
23	X	X	X	X	X	X
24	12,588.0	2.2658	11,804.5	2.1248	13,300.5	2.3941

19	9,894.5	1.7810	11,995.5	2.1592	9,682.5	1.7428
20	9,99[illegible]	1.7991	12,000.0	2.1600	9,777.5	1.7600
21	9,878.5	1.7781	12,027.5	2.1650	9,712.0	1.7482
22	X	X	X	X	X	X
23	9,804.0	1.7647	11,808.5	2.1255	9,833.0	1.7699
24	10,025.0	1.8045	11,756.0	2.1161	10,331.0	1.8596
25	10,004.5	1.8008	11,809.0	2.1256	9,767.5	1.7582
26	9,926.5	1.7868	12,463.0	2.2433	9,647.5	1.7366
27	10,680.5	1.9225	11,428.0	2.0570	10,105.0	1.8189
28	10,365.0	1.8657	11,382.0	2.0488	10,024.0	1.8043
29	X	X	X	X	X	X
30	9,695.0	1.7451	11,651.0	2.0972	9,772.0	1.7590
31	10,119.5	1.8215	11,962.0	2.1532	9,783.0	1.7609
September 1	10,034.0	1.8061	11,986.5	2.1576	9,982.0	1.7968
2	10,056.0	1.8101	12,037.0	2.1667	9,754.0	1.7557
3	9,844.0	1.7719	12,364.0	2.2255	9,706.0	1.7471
4	9,742.0	1.7536	11,747.5	2.1146	9,750.5	1.7551
5	10,335.5	1.8604	11,654.0	2.0977	9,802.0	1.7644
6	9,712.0	1.7482	12,040.5	2.1673	9,768.0	1.7582
7	9,460.0	1.7028	11,819.0	2.1274	9,704.5	1.7468
8	9,467.0	1.7041	11,989.5	2.1581	9,832.0	1.7698
9	9,518.0	1.7132	11,728.5	2.1111	9,894.5	1.7810
10	9,611.5	1.7301	11,660.5	2.0989	9,457.0	1.7023
Bed Rest II ended 2 p.m.						
11	9,880.5	1.7785	11,811.5	2.1261	10,141.0	1.8254
12	9,922.5	1.7861	11,923.0	2.1461	10,737.0	1.9327
13	10,251.0	1.8452	12,017.5	2.1632	10,207.5	1.8374
14	10,473.0	1.8851	12,142.5	2.1856	10,024.5	1.8044
15	X	X	X	X	X	X
16	10,465.5	1.8838	12,099.0	2.1778	10,209.5	1.8377
17	10,587.0	1.9057	12,082.0	2.1748	10,131.0	1.8236
18	10,441.5	1.8795	12,376.5	2.2278	10,082.5	1.8148
19	10,067.5	1.8122	11,987.5	2.1578	10,164.0	1.8295
20	10,155.5	1.8280	12,363.0	2.2253	10,042.5	1.8076
21	10,591.0	1.9064	X	X	X	X
22	10,076.5	1.8138	12,500.0	2.2500	10,576.5	1.9038
23	X	X	11,951.5	2.1513	10,184.0	1.8331
24	10,334.0	1.8601	12,601.0	2.2682	10,759.0	1.9366

10	X	X	X	[illegible]	[illegible]	[illegible]
11	9,783.[illegible]	1.7609	11,961.0	2.1530	9,908.0	1.7834
12	9,950.5	1.7911	11,860.5	2.1349	9,877.5	1.7780
13	9,698.0	1.7456	11,707.0	2.1073	9,847.0	1.7725
14	9,507.5	1.7114	11,768.5	2.1183	9,856.5	1.7742
15	X	X	X	X	X	X
16	9,674.0	1.7413	11,687.0	2.1037	9,849.0	1.7728
17	9,853.5	1.7736	11,605.5	2.0890	9,886.0	1.7795
18	9,765.0	1.7577	11,507.0	2.0713	9,722.0	1.7500
19	10,205.5	1.8370	11,483.5	2.0670	9,642.5	1.7356
20	10,139.0	1.8250	11,361.5	2.0451	9,875.0	1.7775
21	10,002.5	1.8004	11,451.5	2.0613	9,727.0	1.7509
22	9,986.0	1.7975	11,406.0	2.0531	9,828.5	1.7691
23	9,931.5	1.7877	11,421.0	2.0558	9,810.5	1.7659
24	9,857.0	1.7743	11,768.5	2.1183	9,717.0	1.7491
25	9,761.5	1.7571	11,368.0	2.0462	9,840.5	1.7713
26	9,943.0	1.7897	11,576.5	2.0838	9,714.0	1.7485
27	9,804.5	1.7648	11,682.0	2.1028	9,648.0	1.7366
28	9,695.0	1.7451	11,423.5	2.0562	9,599.5	1.7279
29	9,540.5	1.7173	11,761.0	2.1170	9,652.0	1.7374
30	9,574.0	1.7233	11,350.5	2.0431	9,687.0	1.7437
Bed Rest I ended at 2 p.m. (No Exercise during this period)						
31	9,430.5	1.6975	11,802.0	2.1244	9,533.5	1.7160
August 1	10,356.0	1.8641	12,051.5	2.1693	9,802.5	1.7644
2	10,461.5	1.8831	12,002.0	2.1604	9,974.0	1.7953
3	10,674.0	1.9213	12,101.5	2.1783	10,163.5	1.8294
4	X	X	X	X	X	X
5	10,439.5	1.8791	12,564.5	2.2616	10,125.0	1.8225
6	X	X	X	X	X	X
7	10,651.0	1.9172	12,674.5	2.2814	10,222.0	1.8400
8	10,805.5	1.9450	12,722.0	2.2900	10,579.0	1.9042
9	11,237.0	2.0227	12,498.0	2.2496	10,896.5	1.9614
10	X	X	X	X	X	X
11	X	X	X	X	X	X
12	10,758.0	1.9364	12,633.0	2.2739	10,511.5	1.8921
13	10,919.5	1.9655	11,789.5	2.1221	10,334.0	1.8601
Bed Rest II began (Exercise) 2 p.m.						
14	10,007.0	1.8013	11,598.0	2.0876	10,473.5	1.8852
15	10,919.5	1.9655	11,784.0	2.1211	10,005.0	1.8009
16	10,182.5	1.8328	11,848.0	2.1326	10,268.5	1.8483
17	10,163.0	1.8293	11,610.0	2.0898	10,342.0	1.8616
18	10,348.0	1.8626	11,774.5	2.1194	10,278.5	1.8501
19	9,894.5	1.7810	11,995.5	2.1592	9,682.5	1.7428
[illegible]	[illegible]	[illegible]	[illegible]	2.1600	9,777.5	1.7600

	Counts	Wedge Equivalency	Counts	Wedge Equivalency	Counts	Wedge Equivalency
June 3, 1968	10,507.0	1.8913	12,712.5	2.2882	10,520.0	1.8936
4	10,382.5	1.8688	12,527.5	2.2550	10,484.0	1.8871
5	X	X	X	X	X	X
6	X	X	X	X	X	X
7	10,391.0	1.8704	11,864.5	2.1356	10,634.5	1.9142
8	X	X	X	X	X	X
9	X	X	X	X	X	X
10	10,480.0	1.8864	12,862.0	2.3152	10,896.0	1.9613
11	10,449.5	1.8809	12,249.0	2.2048	10,901.5	1.9623
12	X	X	X	X	X	X
13	10,377.0	1.8679	12,364.00	2.2255	10,801.0	1.9442
14	X	X	X	X	X	X
15	10,424.5	1.8764	11,757.5	2.1164	10,300.5	1.8541
16	X	X	X	X	X	X
17	10,281.0	1.8506	12,215.0	2.1987	10,579.0	1.9042
18	10,193.5	1,8348	12,732.5	2.2918	10,973.5	1.9752
19	X	X	X	X	X	X
20	10,195.5	1.8352	12,294.5	2.2130	10,674.0	1.9213
21	10,325.0	1.8585	11,984.5	2.1572	11,216.0	2.0189
22	X	X	X	X	X	X
23	X	X	X	X	X	X
24	10,522.0	1.8940	12,419.5	2.2355	11,099.5	1.9979
25	X	X	X	X	X	X
26	10,323.0	1.8581	12,209.0	2.1976	10,819.0	1.9474
27	X	X	X	X	X	X
28	11,317.0	2.0371	12,236.0	2.2025	10,614.5	1.9106
29	X	X	X	X	X	X
30	X	X	X	X	X	X
July 1	10,481.5	1.8867	12,032.5	2.1658	10,975.5	1.9756
Bed Rest I began, 2 p.m., no Exercise during the Bed Rest						
2	X	X	X	X	X	X
3	10,207.0	1.8373	[illegible],058.0	2.1704	10,450.5	1.8811
4	10,006.5	1.8012	[illegible],143.5	2.1858	10,301.0	1.8542
5	X	X	X	X	X	X
6	10,000.0	1.8000	[illegible],048.0	2.1686	9,833.5	1.7700
7	9,964.0	1.7935	[illegible],180.5	2.1925	9,892.0	1.7806
8	9,853.5	1.7736	[illegible],087.0	2.1757	10,003.5	1.8006
9	X	X	X	X	10,103.0	1.8185
10	X	X	X	X	X	X
11	9,783.0	1.7609	[illegible],961.0	2.1530	9,908.0	1.7834
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	9,877.5	1.7780

TABLE IV

BONE DENSITY VALUES OF CENTRAL SECTION OF OS CALCIS ON DESIGNATED DAYS OF THE STUDY

(Subjects who Exercised "at will" during Bed Rest II)

Date	Subject GG		Subject AA		Subject EE	
	Integrator Counts	A x 0.00018 Calibration Wedge Equivalency	Integrator Counts	A x 0.00018 Calibration Wedge Equivalency	Integrator Counts	A x 0.00018 Calibration Wedge Equivalency
June 3, 1968	10,507.0	1.8913	12,7[illegible]2.5	2.2882	10,520.0	1.8936
4	10,382.5	1.8688	12,5[illegible]7.5	2.2550	10,484.0	1.8871
5	X	X	X	X	X	X
6	X	X	X	X	X	X
7	10,391.0	1.8704	11,864.5	2.1356	10,634.5	1.9142
8	X	X	X	X	X	X
9	X	X	X	X	X	X
10	10,480.0	1.8864	12,[illegible]62.0	2.3152	10,896.0	1.9613
11	10,449.5	1.8809	12,[illegible]49.0	2.2048	10,901.5	1.9623
12	X	X	X	X	X	X
13	10,377.0	1.8679	12,[illegible]64.00	2.2255	10,801.0	1.9442
14	X	X	X	X	X	X
15	10,424.5	1.8764	11,757.5	2.1164	10,300.5	1.8541
16	X	X	X	X	X	X
[illegible]	[illegible]	[illegible]	12,215.0	2.1987	10,579.0	1.9042

CHANGES IN BONE DENSITY OF SECTIONS OF THE FOOT

The first bed rest period, when no exercise program was followed, was compared for each subject in the study as to the per cent change in each anatomic site in the foot in comparison with the corresponding section during the second bed rest, with exercise. The same was repeated for the hand.

The former skeletal sites included: (a) the central os calcis section; (b) multiple sections across approximately 60 per cent of the os calcis; and (c) a central section across the talus.

CHANGES IN BONE DENSITY OF SECTIONS OF THE HAND, WRIST, AND LOWER ARM

The series of sites across the various parts of the hand, wrist, and distal arm included: (a) hand phalanx 4/2; (b) a diagonal section across the capitate; and (c) sections across the distal ends of the radius and ulna.

CENTRAL SECTION OF OS CALCIS

Subjects who Exercised Regularly

Subject BB

Per cent change during Bed Rest I . . . . . . -10.70

Per cent change during Bed Rest II . . . . . - 2.95

Subject FF

Per cent change during Bed Rest I . . . . . . -15.70

Per cent change during Bed Rest II . . . . . - 0.21

Subject HH

Per cent change during Bed Rest I . . . . . . -12.30

Per cent change during Bed Rest II . . . . . - 3.20

Subjects who Exercised "At Will"

Subject AA

Per cent change during Bed Rest I . . . . . . - 5.70

Per cent change during Bed Rest II . . . . . - 7.80

Subject EE

Per cent change during Bed Rest I . . . . . . -10.20

Per cent change during Bed Rest II . . . . . -10.03

Subject GG

Per cent change during Bed Rest I . . . . . . - 8.70

Per cent change during Bed Rest II . . . . . -11.98

MULTIPLE SECTIONS OF OS CALCIS

Subjects who Exercised Regularly

Subject BB

Per cent change during Bed Rest I . . . . . . - 9.35

Per cent change during Bed Rest II . . . . . - 3.10

Subject FF

Per cent change during Bed Rest I	-12.05
Per cent change during Bed Rest II	- 0.70

Subject HH

Per cent change during Bed Rest I	-12.00
Per cent change during Bed Rest II	- 3.50

Subjects who Exercised "At Will"

Subject AA

Per cent change during Bed Rest I	- 6.00
Per cent change during Bed Rest II	- 4.94

Subject EE

Per cent change during Bed Rest I	- 8.87
Per cent change during Bed Rest II	-10.00

Subject GG

Per cent change during Bed Rest I	- 8.75
Per cent change during Bed Rest II	-12.05

CENTRAL SECTION OF TALUS

Subjects who Exercised Regularly

Subject BB

Per cent change during Bed Rest I	- 5.46
Per cent change during Bed Rest II	- 2.26

Subject FF

Per cent change during Bed Rest I . . . . - 8.33

Per cent change during Bed Rest II . . . . . . - 2.86

Subject HH

Per cent change during Bed Rest I . . . . - 8.73

Per cent change during Bed Rest II . . . . . . - 2.99

Subjects who Exercised "At Will"

Subject AA

Per cent change during Bed Rest I . . . . - 7.60

Per cent change during Bed Rest II . . . . . . - 2.98

Subject EE

Per cent change during Bed Rest I . . . . - 8.68

Per cent change during Bed Rest II . . . . . . - 7.52

Subject GG

Per cent change during Bed Rest I . . . . - 9.60

Per cent change during Bed Rest II . . . . . . - 6.98

MULTIPLE SECTIONS OF HAND PHALANX 4-2

Subjects who Exercised Regularly

Subject BB

Per cent change during Bed Rest I . . . . - 8.57

Per cent change during Bed Rest II . . . . . . - 4.03

Subject FF

Per cent change during Bed Rest I . . . . . . - 6.52

Per cent change during Bed Rest II . . . . . - 3.10

Subject HH

Per cent change during Bed Rest I . . . . . . - 7.35

Per cent change during Bed Rest II . . . . . - 3.24

Subjects who Exercised "At Will"

Subject AA

Per cent change during Bed Rest I . . . . . . - 6.92

Per cent change during Bed Rest II . . . . . - 5.09

Subject EE

Per cent change during Bed Rest I . . . . . . - 6.82

Per cent change during Bed Rest II . . . . . - 5.96

Subject GG

Per cent change during Bed Rest I . . . . . . - 8.72

Per cent change during Bed Rest II . . . . . - 6.01

DIAGONAL SECTION ACROSS CAPITATE

Subjects who Exercised Regularly

Subject BB

Per cent change during Bed Rest I . . . . . . - 8.81

Per cent change during Bed Rest II . . . . . - 2.03

Subject FF

Per cent change during Bed Rest I . . . . . . - 7.77

Per cent change during Bed Rest II . . . . . - 0.40

Subject HH

Per cent change during Bed Rest I . . . . . . - 7.01

Per cent change during Bed Rest II . . . . . + 0.10

Subjects who Exercised "At Will"

Subject AA

Per cent change during Bed Rest I . . . . . . - 8.07

Per cent change during Bed Rest II . . . . . - 4.69

Subject EE

Per cent change during Bed Rest I . . . . . . - 9.32

Per cent change during Bed Rest II . . . . . - 7.38

Subject GG

Per cent change during Bed Rest I . . . . . . - 6.72

Per cent change during Bed Rest II . . . . . - 5.06

SECTION ACROSS DISTAL END OF RADIUS

Subjects who Exercised Regularly

Subject BB

Per cent change during Bed Rest I . . . . . . - 6.41

Per cent change during Bed Rest II . . . . . - 2.29

Subject FF

Per cent change during Bed Rest I . . . . - 5.47

Per cent change during Bed Rest II . . . . . . - 2.05

Subject HH

Per cent change during Bed Rest I . . . . - 6.51

Per cent change during Bed Rest II . . . . . . - 3.06

Subjects who Exercised "At Will"

Subject AA

Per cent change during Bed Rest I . . . . - 8.93

Per cent change during Bed Rest II . . . . . . - 5.17

Subject EE

Per cent change during Bed Rest I . . . . - 5.74

Per cent change during Bed Rest II . . . . . . - 7.55

Subject GG

Per cent change during Bed Rest I . . . . - 5.89

Per cent change during Bed Rest II . . . . . . - 4.17

SECTION ACROSS DISTAL END OF ULNA

Subjects who Exercised Regularly

Subject BB

Per cent change during Bed Rest I . . . . - 7.17

Per cent change during Bed Rest II . . . . . . - 1.80

Subject FF

Per cent change during Bed Rest I . . . . . . -10.83

Per cent change during Bed Rest II . . . . . - 3.05

Subject HH

Per cent change during Bed Rest I . . . . . . - 9.49

Per cent change during Bed Rest II . . . . . - 2.85

Subjects who Exercised "At Will"

Subject AA

Per cent change during Bed Rest I . . . . . . - 6.35

Per cent change during Bed Rest II . . . . . - 4.08

Subject EE

Per cent change during Bed Rest I . . . . . . - 8.75

Per cent change during Bed Rest II . . . . . - 9.60

Subject GG

Per cent change during Bed Rest I . . . . . . - 7.50

Per cent change during Bed Rest II . . . . . - 6.08

The data given above concerning the skeletal density changes in the evaluated anatomic sites showed in general that the losses during Bed Rest I were greater than those for Bed Rest II for the subjects who exercised regularly during the second bed rest, with the same not found for those who exercised only when they decided to do so. The differences between Bed Rest I and Bed Rest II differed in the various skeletal sites, although the regular exercise group generally showed reductions in the bone density loss in all sites greater than did the "at will" group.

Central Os Calcis Section. There were marked differences between the changes in bone density values of the central or "conventional" section of the os calcis from one bed rest period to the other of those who exercised regularly and those who exercised "at will". The regular exercise group experienced marked reductions in bone density loss between the first bed rest with no exercise and the second bed rest with regular exercise four times a day.

Those who exercised "at will" during the second bed rest, and in common with those who exercised regularly during this period, not at all during Bed Rest I, showed very little change in bone density of the central os calcis section from one bed rest period to the other.

Multiple Os Calcis Sections. The total of the multiple os calcis sections, which covered this bone entirely from the point where it was overlapped by another bone to its distal perimeter, was similar to the central os calcis section in the bone density changes from Bed Rest I to Bed Rest II. The subjects who exercised regularly experienced marked reductions in bone density loss in Bed Rest II, whereas one subject, Subject AA showed a minor loss reduction, with the other two subjects losing slightly more in Bed Rest II than in Bed Rest I.

Central Section of the Talus. The men who exercised regularly showed marked decreases in bone density losses of the talus between Bed Rest I and Bed Rest II in the central section of the talus. The irregular exercise group showed some reduction in bone density in this skeletal site, although this was far less for all three men than for those who exercised regularly.

Multiple Sections of Hand Phalanx 4/2. For those who exercised regularly, the loss in bone density of hand phalanx 4/2, covering the entire hand phalanx 4/2 was reduced during Bed Rest II, approximately to one-half that experienced by the same subjects during Bed Rest I. For the "at will" exercise group, on the other hand, the loss was reduced by a much lesser proportion.

The smaller reduction in the bone density loss from this skeletal site, even for the men who exercised regularly, probably resulted from the fact that the second phalanx of the fourth finger was not involved in the exercise with the Exer-Grip to the extent of pulling muscles attached to this bone to any great extent.

Diagonal Section across the Capitate. Marked reductions in bone density of the diagonal section assessed across the capitate was found in the subjects who exercised regularly, with much lesser reductions in this skeletal location in those who exercised only when they wished to do so. It is believed that, in squeezing the Exer-Grip, the wrist tended to be bent at an angle a part of the time during the exercise period, which would activate the muscles attached to wrist bones.

Skeletal Section across the Distal End of the Radius. Of the men who exercised four times a day regularly, there were distinct lowering of bone density losses in a section across the distal end of the radius in all subjects in going from Bed Rest I to Bed Rest II. On the other hand, two of the men who exercised "at will" experienced minor losses in Bed Rest II in comparison with Bed Rest I, with one man exhibiting an excrease in bone density loss during the second bed rest period.

Skeletal Section across the Distal End of the Ulna. The regular exercise group exhibited very marked reductions in bone density losses in the distal end of the ulna during Bed Rest II in comparison with Bed Rest I. Again, two of the men in the exercise "at will" group showed only minor reduction in skeletal losses in this bone, whereas one man lost slightly more during Bed Rest II than in Bed Rest I in this skeletal site.

It appears very probable that during both the isometric portion of the exercise program when the arms were deeply involved in the exercise routine, and in the hand gripping part of the program, the wrist and lower arms experienced a distinct pull on the muscles attached to the wrist and the arms.

EXCRETION OF CALCIUM

As has been noted, numerous chemical tests were made on blood and urine of the subjects of this study by other investigators in these laboratories. The work which was most closely associated with the investigation described herein was that of Van Zandt (84), which involved the analysis of calcium and phosphorus in urine and feces. This work, which was continued throughout the five periods of the study, supported the findings of the author.

The following is quoted from the work of this investigator:

"Statistical analyses of the data showed that the quantity of calcium excreted during Bed Rest I surpassed that during Bed Rest II by a difference which was highly significant for those subjects who exercised regularly, while those exercising 'at will' excreted less calcium during Bed Rest II by a smaller, though slightly significant, amount. These results demonstrated the finding that, with subjects on a uniform diet which eliminated diet as a variable, exercise during a highly supervised horizontal bed rest reduced calcium loss in the urine markedly when regular programmed exercise was followed, and to a certain extent even if the exercise was not routine.

"A greater proportion of the calcium was excreted in the feces than in the urine in all subjects, as has been found in other studies of this series.

Comparison of Urinary Calcium Excretion during the Equilibration and Bed Rest I Periods

Regular Exercise Group

"Subject BB. When the amount of urinary calcium excreted by Subject BB during the initial Equilibration Period was compared with that lost in the urine during Bed Rest I, a greater quantity of calcium appeared during the immobilization period than during the previous ambulatory period in this subject, by a difference which was highly significant ($P<0.001$).

"Subject FF. During Bed Rest I, the urinary calcium excretion by Subject FF was greater than that of the Equilibration Period, with a difference which was distinctly significant ($P<0.02$).

"Subject HH. The amount of calcium appearing in the urine of Subject HH during Bed Rest I was greater per unit of time than that excreted during the initial Ambulatory Period, with a difference which was highly significant ($P<0.001$).

"At Will" Exercise Group

"Subject AA. Although Subject AA excreted somewhat more urinary calcium during the first Bed Rest than during the initial Equilibration Period, the difference was not statistically significant.

"Subject EE. A comparison of the calcium excreted in the urine of Subject EE during the first Ambulatory Period with that of Bed Rest I shows that the quantity during the Bed Rest was higher than that during the Ambulatory Period by a difference which was statistically significant ($P < 0.05$).

"Subject GG. More urinary calcium was excreted during Bed Rest I by Subject GG than in the initial Equilibration Period, but the difference was not significant in this case.

"All Subjects. When the data for the subjects who exercised regularly during the second Bed Rest were pooled together for the comparison of urinary calcium during the Equilibration Period and Bed Rest I, Table VII, Part G shows that the Bed Rest Period surpassed the Equilibration Ambulatory Period by a highly significant difference ($P < 0.001$).

"When the data on the 'at will' exercise groups likewise were pooled, the calcium excretion in the urine during the recumbency of Bed Rest I surpassed that of the initial Ambulatory Period by a distinctly significant difference ($P < 0.01$).

Comparison of Bed Rest I with Bed Rest II for Urinary Calcium Excretion

Regular Exercise Group

"Subject BB. There was no statistically significant difference between the quantity of urinary calcium excreted by this subject during the two Bed Rest Periods, although the quantity during Bed Rest I was slightly higher than that during Bed Rest II.

"Subject FF. Subject FF, on the other hand, showed a decrease in calcium loss in the urine during Bed Rest II when compared with Bed Rest I, the difference being highly significant ($P < 0.001$).

"Subject HH. The urinary calcium excretion of this subject showed a slight decrease during Bed Rest II in comparison with Bed Rest I, but the difference between the two Bed Rest Periods was not significant.

"At Will" Exercise Group

"Subject AA. Although this subject, too, showed a decreased urinary calcium loss during Bed Rest II over Bed Rest I, the difference was too small to be statistically significant. This subject exercised more consistently than did either of the other Bed Rest subjects.

"Subject EE. A comparison of Bed Rest Periods for Subject EE indicated that a somewhat greater loss had occurred in urinary calcium excretion during Bed Rest I than in Bed Rest II, with a difference which was slightly significant.

"Subject GG. This subject excreted a higher amount of urinary calcium during Bed Rest I than during Bed Rest II, with a difference which was distinctly significant.

"All Subjects. When the total urinary calcium excretion data for the three participants in the study who exercised regularly were combined together, the 't' test showed that the quantity of this element excreted during Bed Rest I surpassed that during Bed Rest II by a difference which was highly significant ($P<0.001$). It will be recalled that, during Bed Rest II, exercise was taken routinely on a programmed basis by three men, and at irregular times when called for by the other three.

"Of the three subjects who exercised 'at will', the calcium excretion in the urine during Bed Rest I was shown in a pooled data analysis to have surpassed that excreted during Bed Rest II by a difference which was significant ($P<0.01$). This is consistent with the extent of exercising of the respective sub-groups.

Comparison of Total Calcium Excretion (Urinary and Fecal) during the Two Bed Rest Periods

"The Summary which follows gives the data on mean daily calcium excretion in the urine and feces during Bed Rest I and Bed Rest II, respectively, for each participant in the study.

"Table IV shows that a greater proportion of the calcium was excreted in the feces than in the urine in all subjects. Because the subjects were on a low residue diet, their fecal excretions were very irregular.

"Figure 9 shows, by means of bar graphs, a comparison of the mean levels of total calcium (urinary and fecal) excreted by the six subjects in the study. It should be noted that the only man in which the excretion of calcium during Bed Rest II slightly exceeded that during Bed Rest I was Subject EE. This subject exercised the least of

TABLE V

[from Van Zandt (84)]

MEAN OF DAILY URINARY AND FECAL CALCIUM EXCRETION BY EACH INDIVIDUAL SUBJECT IN THE STUDY DURING THE TWO RESPECTIVE BED REST PERIODS

(Milligrams per Day)

Subject	BED REST I			BED REST II		
	Urinary Excretion	Fecal Excretion	Total Excretion	Urinary Excretion	Fecal Excretion	Total Excretion
BB	296	814	1110	273	598	871
FF	305	825	1130	235	850	1085
HH	269	900	1169	253	768	1021
GG	319	874	1193	282	701	983
AA	417	676	1093	383	647	1030
EE	412	769	1179	341	919	1260

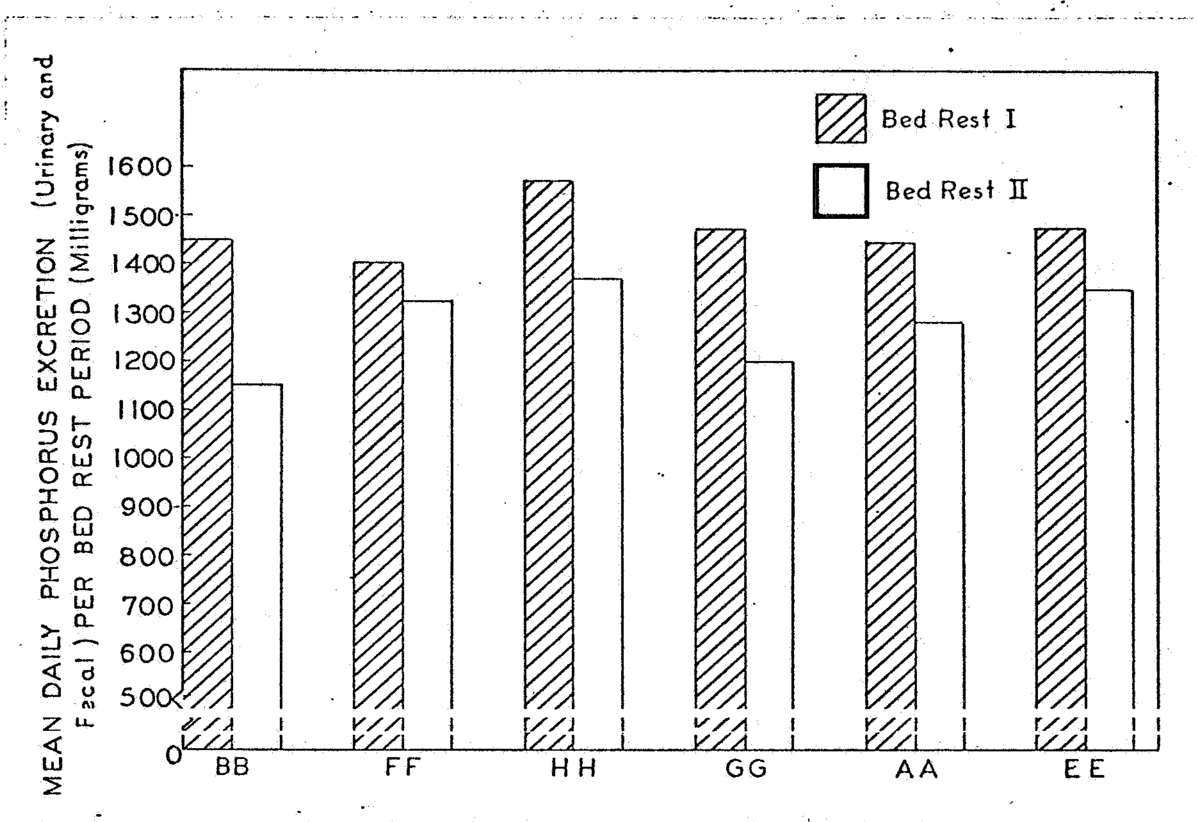


Figure 9. COMPARATIVE EXCRETION OF TOTAL CALCIUM (URINARY AND FECAL) DURING BED REST I AND BED REST II FOR EACH PARTICIPATING SUBJECT

[from Van Zandt (84)]

of any of the 'at will' group, with a total number of minutes expended in this activity totalling about one-third of the time expended by those who exercised regularly. This subject was shown to have excreted somewhat more urinary calcium during Bed Rest I than during Bed Rest II, with the difference statistically significant. In view of the small amount of time which he spent exercising, this was somewhat surprising. When his fecal excretion was included, however, the results from the two sources of calcium showed very slightly more mineral loss during the second than the first Bed Rest.

Comparison of Total Calcium Excretion during Bed Rest I and Bed Rest II by the Two Exercise Groups

"The subjects exercising 'at will' excreted more calcium in the urine during Bed Rest II than did those exercising regularly. Five of the six subjects lost less calcium during Bed Rest II, with exercise, than during Bed Rest I, without exercise. The one subject who excreted more calcium during the latter period was the one who exercised least.

"Table V shows the total excretion of urinary and fecal calcium during the two bed rest periods by the regular and the 'at will' exercise groups.

EXCRETION OF PHOSPHORUS

As in the case of the calcium urinary excretion data, the phosphorus findings will be given first for the individual subjects, with

TABLE VI

[from Van Zandt (84)]

COMPARISON OF TOTAL CALCIUM EXCRETION DURING BED REST I AND BED REST II BY THE REGULAR AND THE "AT WILL" EXERCISE GROUPS

Populations Compared	Means (milli-grams)	Standard Deviation	"t" Value	Probability
During Bed Rest I				
Regular Exercisers	1136	71	1.3337	N.S.
"At Will" Exercisers	1155	110		
During Bed Rest II				
Regular Exercisers	939	174	6.0931	$P<0.001$
"At Will" Exercisers	1091	145		

findings as shown by the analysis of pooled data for the two sub-groups of subjects following thereafter.

Comparison of Urinary Phosphorus Excretion during the Equilibration and Bed Rest I Periods

Regular Exercise Group

"Subject BB. When the amount of urinary phosphorus excreted by Subject BB during the Initial Equilibration Period was compared with that lost in the urine during Bed Rest I, an increase was shown during immobilization. The difference between the two values, however, was barely significant ($P<0.10$).

"Subject FF. There was no statistically significant difference in urinary phosphorus excretion when a comparison was made between the Initial Equilibration Period and Bed Rest I for this subject.

"Subject HH. The urinary phosphorus loss for Subject HH increased during Bed Rest I in comparison with that of the Initial Equilibration Period, with the difference highly significant ($P<0.001$).

'At Will' Exercise Group

"Subject AA. No significant difference in urinary phosphorus loss was evidenced by Subject AA when the Initial Equilibration and Bed Rest I Periods were compared.

"Subject EE. The amount of phosphorus lost in the urine of Subject EE showed no significant change during the Initial Ambulatory and the first Immobilization Periods.

"Subject GG. Likewise there was no change in the quantity of urinary phosphorus eliminated during the Initial Equilibration Period and Bed Rest I for this subject.

"All Subjects. When the data for the three subjects who exercised regularly were pooled for the purpose of statistical analysis, an increase in urinary phosphorus excretion was shown in Bed Rest I in comparison with the Initial Equilibration Period. The difference in phosphorus loss between the two periods was distinctly significant ($P<0.01$).

"Similarly, when the data were pooled for the purpose of finding whether there was a significant difference between losses of phosphorus during Bed Rest I and the previous Equilibration Ambulatory Period for the 'at will' exercise group, there was not a statistically significant difference between the levels of secretion of this entity between the two periods, although the value excreted during Bed Rest I surpassed that of the previous Ambulatory Period to some extent.

Comparison of Bed Rest I with Bed Rest II for Urinary Phosphorus Excretion

Regular Exercise Group

"Subject BB. The quantity of phosphorus excreted in the urine by Subject BB decreased during Bed Rest II over that of Bed Rest I. The difference between the two was statistically significant ($P<0.05$). This man engaged in regular exercise for a total of 2,194 minutes during the second Bed Rest Period, which was instrumental in causing the decrease in excretion of this mineral component of bone.

"Subject FF. When Bed Rest I was compared to Bed Rest II for Subject FF, the urinary phosphorus loss was shown to have decreased during the second period. The difference, however, had a low level of significance ($P<0.10$). This subject, who exercised regularly during Bed Rest II for a total of 2,181 minutes, experienced some digestive trouble during the study.

"Subject HH. The statistics for this subject indicated that Subject HH excreted more urinary phosphorus during Bed Rest I than during Bed Rest II, the difference being highly significant ($P<0.001$). The amount of regular exercise in which this subject engaged during Bed Rest II totalled 1,913 minutes, and its effect is clearly seen in the large decrease in phosphorus excreted during this time.

"At Will" Exercise Group

"Subject AA. A comparison of Bed Rest I with Bed Rest II revealed the finding that Subject AA excreted less phosphorus in the urine during Bed Rest II than during Bed Rest I. This decrease represented a difference of distinct statistical significance ($P<0.01$). This subject exercised 'at will' during the second Bed Rest Period for a total of 577 minutes, and even this relatively small amount of time is reflected in a decrease in urinary phosphorus excretion. It should be noted that he spaced his exercise periods with some regularity, which can not be said of the others in the 'at will' group.

"Subject EE. The amount of urinary phosphorus excreted by Subject EE during Bed Rest II was less than that of Bed Rest I, the difference between the two amounts being statistically significant ($P<0.02$). The small total of 182 minutes of exercise for this subject is reflected in a decrease of phosphorus lost through the urine.

"Subject GG. The amount of phosphorus excreted in the urine by Subject GG decreased during Bed Rest II in comparison with Bed Rest I. The difference was sufficiently great to be highly significant ($P<0.001$). Although this subject discontinued regular exercise after seven days, and thereafter engaged in exercise only on an 'at will' basis, the amount achieved during this period was 855 minutes. Its effect is reflected in the large decrease in phosphorus loss during this time.

"All Subjects. When the data were pooled so that an overall comparison could be made between Bed Rests I and II for the three subjects who exercised regularly, it was found that phosphorus excretion during Bed Rest I (no exercise) greatly exceeded that of Bed Rest II ($P<0.001$).

"The same level of difference also was found between Bed Rest I and Bed Rest II for the 'at will' exercisers.

Comparison of Total Phosphorus Excretion (Urinary and Fecal) during the Two Bed Rest Periods

"Table VI gives the data on mean daily phosphorus excretion in the urine and feces during Bed Rest I and Bed Rest II for each

TABLE VII

[from Van Zandt (84)]

MEAN OF DAILY URINARY AND FECAL PHOSPHORUS EXCRETION BY EACH INDIVIDUAL SUBJECT IN THE STUDY DURING THE TWO RESPECTIVE BED REST PERIODS

(Milligrams per Day)

Subject	BED REST I			BED REST II		
	Urinary Excretion	Fecal Excretion	Total Excretion	Urinary Excretion	Fecal Excretion	Total Excretion
BB	1028	427	1455	939	333	1272
FF	976	428	1404	895	430	1325
HH	1054	516	1570	919	449	1368
AA	1027	417	1444	888	395	1283
EE	1049	427	1476	906	439	1345
GG	1075	398	1473	863	332	1195

participant in the study.

"In contrast with the calcium excretion pattern, the major portion of the phosphorus which is excreted is found in the urine. The distribution between urine and feces differs for the various individuals.

"Table VII separates the regular exercise group from those who exercised when they desired to do so. No statistically significant difference was found between the two groups in total phosphorus excretion either during Bed Rest I or II.

"As in the case of calcium, the excretion of phosphorus during Bed Rest I surpassed that during Bed Rest II for all subjects, whether they exercised regularly or 'at will'. This is shown graphically in Figure 10."

TABLE VIII

[from Van Zandt (84)]

COMPARISON OF TOTAL PHOSPHORUS EXCRETION DURING BED REST I AND BED REST II BY THE REGULAR AND THE "AT WILL" EXERCISE GROUPS

Populations Compared	Means (milli-grams)	Standard Deviation	"t" Value	Probability
During Bed Rest I				
Regular Exercisers	1476	186	0.5180	N.S.
"At Will" Exercisers	1459	240		
During Bed Rest II				
Regular Exercisers	1284	164	0.3548	N.S.
"At Will" Exercisers	1275	156		

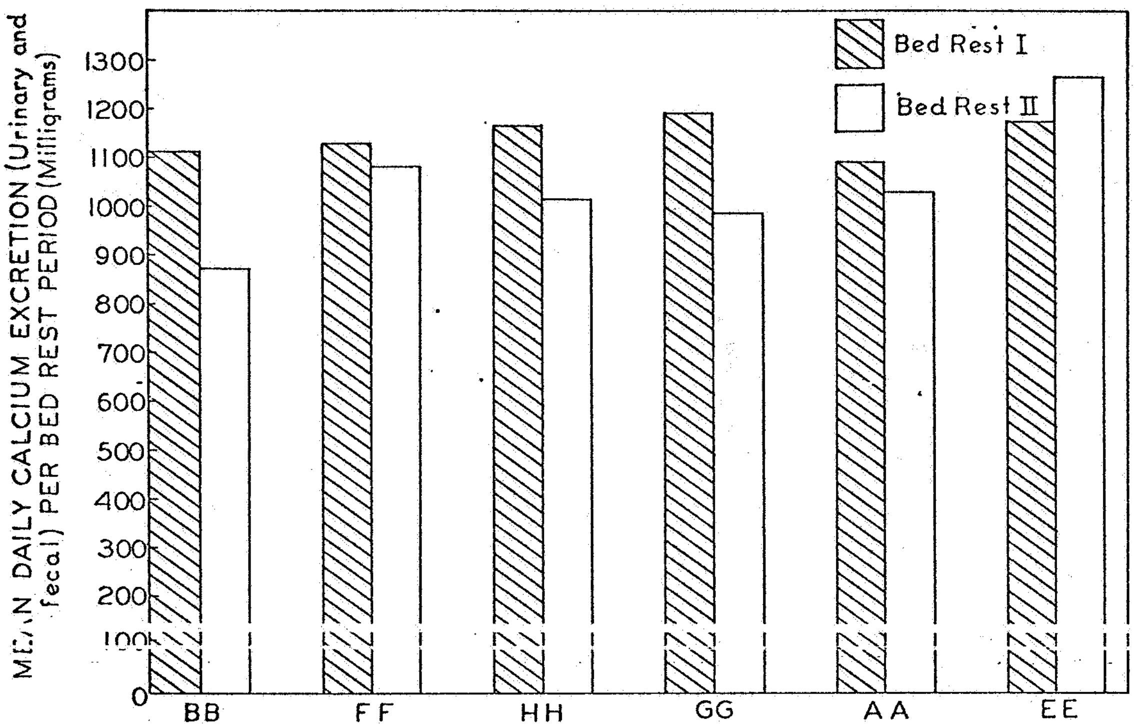


Figure 10. COMPARATIVE EXCRETION OF TOTAL PHOSPHORUS (URINARY AND FECAL) DURING BED REST I AND BED REST II FOR EACH PARTICIPATING SUBJECT

[from Van Zandt (84)]

DISCUSSION

Trueta, Nuffield Professor Emeritus of Orthopedic Surgery at the University of Oxford, in his classical treatise on Studies of the Development and Decay of the Human Frame (85) has stated: "The ability of the osteoblast [in the bone] to synthesize the required protein for its matrix depends on the availability of amino acids and other constituents needed for the synthesis. It has long been known that immobilization, bed rest, and lack of muscle activity are all factors contributing to osteoporosis, a condition of decreased skeletal mass associated with increased porosity. Why this is so has not yet been fully explained, but that the osteoblast-osteocyte syncytium appears to be adversely affected by lack of activity is unquestionable."

In order to throw light on this problem, Trueta and others have carried on animal experiments designed to offer some explanation of the osteoporosis of inactivity.

Geiser and Trueta (51) in a study of Muscle Action, Bone Rarefaction, and Bone Formation, which has been cited and discussed briefly in this report, have carried on an extensive study involving rabbits, from which they concluded that rarefaction of the calcaneum of animals in five experiments occurred soon after the bone was relieved of muscular compressing forces. They found that new bone was generated when the calcaneum again was subjected to the stresses and strains of muscle contraction. These authors concluded further that, in the calcaneum of the rabbit, lack of muscular action seems to be the most important factor inducing osteoporosis. They state also that it is possible that the origin of post-traumatic osteoporosis has the same basis.

de Valderrama and Trueta (86) carry the concept of the relation of muscle activity to intra-osseous circulation. They review certain aspects of the investigations of Trueta (85) and of Geiser and Trueta (51) with respect to osteoporosis being caused by the suppression of muscle activity, and they state that these investigators have found that the intra-osseous vessels increased in size and number where bone removal exceeded bone deposition.

The purpose of the investigation of these workers was to study the muscle contribution to the flow of blood through bone. Preliminary observations concerning this study were given by Trueta (87) at the Conference on Bone Biodynamics at the Ford Hospital, Detroit in 1964.

In this study, adult mongrel dogs were used. The effect of muscle contraction on the blood supply of the adjacent bone was investigated by determinations of the intra-osseous pressure in the tibia of the dog during varying conditions of muscle activity.

They found that muscular contraction seems to influence the blood flow in bone in two ways: (a) by the occlusion of the venous outflow, which leads to an increase in intramedullary pressure; and (b) by the rapid emptying of the intra-osseous venous system following muscle relaxation, which causes the intra-osseous pressure to fall.

Harris and Heaney (88), in a review of skeletal renewal and metabolic bone disease, state the following: "The skeleton, containing 99 per cent of the total body calcium, serves two major functions. First of all, it plays an important part in calcium homeostasis, both responding to and contributing to changes in calcium metabolism. Secondly, the structural integrity of the skeleton is essential for normal existence. Fractures, by far the most important abnormality of the skeletal system, occur with increasing frequency in the elderly because of decreasing strength of the skeleton. This weakness is due largely to a reduction in skeletal mass caused by an imbalance between the formation and the resorption of bone. Throughout life, even after cessation of longitudinal growth, cancellous and cortical bone are constantly being replaced by resorption of existing areas and by production of new deposits in microscopic amounts at many sites heterogeneously distributed throughout the skeleton. Changes in this balance between formation and resorption have a critical role in calcium homeostasis and underlie every disease with a notable influence on the adult skeleton."

In the study described in this dissertation, the diet was uniform for all subjects, and was planned with special attention given to the dietary components believed to be essential to the maintenance of skeletal mass, with exercise the only variable. Trueta (1) has discussed not only calcium, phosphorus, and protein as necessary in bone formation, but also polysaccharides, which are important in collagen production, as well as various vitamins. This author emphasizes particularly vitamin A, ascorbic acid, and vitamin D. These are important not only in bone formation during growth, but also in the maintenance of the integrity of the skeleton in adults.

The most extensive work on vitamin A and bone status was done by Mellanby (89), with Mack and associates having carried on various studies on vitamin A and ascorbic acid in connection with bone density status. Vitamin A equivalency in its relation to bone density was included in a study of college women published in 1955 (90) and in unpublished human nutrition studies conducted from 1953 to 1968, which included football players and the male adult men in family groups.

Ascorbic acid tests in relation to bone density have been run in these laboratories on experimental animals (rats and primates), on high school students, and on male adult men in bed rest studies. The data from these studies, undergoing preparation for publication at the present time, show that emphasis should be placed on vitamin A and ascorbic acid, preferably from food sources, when the skeletal density is to be maintained or improved.

The importance of dietary calcium in the maintenance of skeletal homeostasis has been discussed by Lutwok (90), based on work carried out in the Metabolic Disease Branch, National Institute of Arthritis and Metabolic Disease, National Institutes of Health, Bethesda, Maryland. This investigator has summarized his report as follows:

Osteoporosis has been examined as a disorder resulting from a relative nutritional imbalance of calcium, leading to increased bone resorption. In some individuals, this may be due to inadequate dietary intake relative to losses from the body. Other patients are in negative calcium balance despite very high dietary intakes; in these, a relative defect in calcium absorption is postulated, correctable in some instances by the administration of modest amounts of vitamin D. Any therapeutic regimen producing positive balance of calcium decreases the rate of bone resorption and hence, presumably, halts the process of demineralization.

It should be noted in connection with this report that the exercise program developed and used in this study has had a trial in the Apollo missions. From information gained during debriefing sessions and from personal conversations between certain of the astronauts and the director of this study, it is believed that the extent of use of the exercisers by the men during flight was a factor which had a definite relationship to the extent of loss of bone density in the os calcis and talus during this period. The fact that only one astronaut (Cunningham) lost no bone density in the hand, wrist, and distal ends of the radius and ulna during flight is believed to emanate from the habit of this man of flexing the fingers frequently, usually around a tubular object. This practice was not reported by the other two men. Apollo VIII men, on the other hand, have stated to the director of this study that they had very little time to exercise. The comparative changes in skeletal status during flight are shown in the following table.

TABLE IX

Change in Bone Density in the Designated Anatomic Sites-- During Flight

Apollo VII Astronauts

	Schirra	Cunningham	Eisele
Central Os Calcis Section	-5.32	+0.74	+2.27
Multiple Os Calcis Sections	-4.10	+1.19	+0.85
Central Talus Section	-3.60	+1.75	+2.89
Hand Phalanx 4-2 Sections	-9.30	+2.04	-6.50
Capitate Section	-4.07	+3.31	-3.44
Distal Radius Section	-3.25	+3.34	-3.64
Distal Ulna Section	-3.02	+2.12	-3.41

Apollo VIII Astronauts

	Borman	Lovell	Anders
Central Os Calcis Section	-2.13	-6.95	-2.93
Multiple Os Calcis Sections	-7.08	-6.04	-6.50
Central Talus Section	-2.62	-2.81	-3.18
Hand Phalanx 4-2 Sections	-2.19	-2.41	+4.81
Capitate Section	-9.60	-12.11	-6.65
Distal Radius Section	-8.76	-11.06	-11.39
Distal Ulna Section	-6.42	-12.41	-16.17

SUMMARY

In preparation for the Apollo missions, the Nelda Childers Stark Laboratory for Human Nutrition Research, a component of the Research Institute at the Texas Woman's University, was requested by the National Aeronautics and Space Administration, Manned Spacecraft Center, to conduct an investigation in which six healthy adult men would participate, with all variables including diet controlled, except the degree of exercising.

The broad objective of the study was to determine the effects of programmed exercise during highly disciplined horizontal bed rest, with two 28-day bed rest periods conducted and with periods of pre-bed rest equilibration and post-bed rest recovery included. During the first bed rest period no exercise was taken by any subject. Following the intervening equilibration ambulatory period, three of the subjects engaged in the exercise program routinely four times daily during the second recumbency period, while the other three subjects participated in the supervised exercise program only when they desired to do so. The four-day cycle menus contained optimum levels of all nutrients, including calcium which was slightly in excess of one gram daily for the 113 days of the study.

During the periods of bed rest, roentgenograms were made of the left hand and the left foot of each subject according to standardized procedures at the Research Institute's laboratories, approximately once

daily. The "Mack Method" of bone densitometry was used to assess the skeletal status of the subjects on the basis of evaluations of certain specified anatomic sites. The skeletal sites from the foot x-rays of each subject included: (a) the central section of the os calcis; (b) multiple parallel sections across approximately 60 per cent of the os calcis; and (c) the central section of the talus.

From the roentgenograms of the hand the following series of sites were analyzed for each subject: (a) hand phalanx 4-2; (b) a diagonal section across the capitate; (c) a section across the digital end of the radius; and (d) a section across the distal end of the ulna.

Identical tracing paths were followed and similar measurements were made from x-ray to x-ray of the same individual and for all six subjects of this study.

During the 28 days of Bed Rest I, all subjects experienced a decrease in bone density ranging from 5.70 to 15.70 per cent in the standard site—the central section of the os calcis. During the period of Bed Rest II, which followed an interim equilibration period, the losses in the three men who exercised regularly with both isometric and isotonic exercises four times a day declined markedly in all anatomic sites, whereas for the three subjects who exercised only when they desired to do so, the rate of loss continued as in Bed Rest I, actually exceeding that of the previous recumbency period in some cases.

The loss of bone density during Bed Rest I in the central section of the os calcis of each subject, for example, increased in comparison with the pre-bed rest equilibration period by a difference which was highly significant. A statistical comparison of Bed Rest I and Bed Rest II with respect to the bone density of the central os calcis section showed an increase in this bone during the bed rest with exercise for those who exercised regularly which was highly significant. An increase which was slightly significant was found only in one subject of the irregular exercise group.

Bone loss was accompanied by increased urinary clacium excretion which diminished remarkably, although not entirely to pre-bed rest levels, for three men who exercised regularly. The os calcis central section, under conditions of this study, appeared to be a sensitive site for the measurement of changes in bone mineral density.

The findings of this study are in line with the work of other investigators who have found that muscular activity is related to homeostatis in bone, and further that muscular activity is accompanied by intra-osseous blood flow.

The findings of this study also are related to the two space missions in which bone density losses, measured by those in this laboratory, were markedly lowered, the missions of Gemini VII and Apollo VII, with the reduction believed to be related to the exercise which was practiced.

The apparent relationship of exercise to the maintenance of skeletal integrity during recumbency at one G and during orbital flight suggests that emphasis should be placed on this finding in future space missions.

R E F E R E N C E S

1. Mack, Pauline Beery and Paul A. LaChance, Effect of Recumbency and Space Flight on Bone Density, The American Journal of Clinical Nutrition, Volume 20, Number 11:1194 (1967)

2. Mack, Pauline Beery and Effie Boggs Creamer, Comparison of Bone Densitometry and Calcium Metabolism in Two 14-Day Bed Rest Periods With and Without Isometric and Isotonic Exercise, Seventh Semiannual Report to NASA on Grant Number NsG-440 (1966)

3. Vogt, F. B., Pauline Beery Mack, W. G. Beasley, W. A. Spencer, D. Cardus and C. Vallbona, The Effect of Bed Rest on Various Parameters of Physiological Function, Part XII. The Effect of Bed Rest on Bone Mass and Calcium Balance, Bulletin Prepared under Contract Number NAS9-1461 by the Texas Institute for Rehabilitation and Research for the National Aeronautics and Space Administration, NASA CR-182 (1965)

4. Mack, Pauline Beery, Paul A. LaChance, George P. Vose, and Fred B. Vogt, Bone Demineralization of Foot and Hand of Gemini-Titan IV, V, and VII Astronauts during Orbital Flight, The American Journal of Roentgenology, Radium Therapy and Nuclear Medicine, Volume C, Number 3:503 (1967)

5. Mack, Pauline Beery, Fred B. Vogt, Walter W. Gilchrist, and Bill J. Stover, Skeletal Density Tests during the Apollo VII and Apollo VIII Missions, Final Report to the National Aeronautics and Space Administration, Manned Spacecraft Center, from the Texas Woman's University, based on Contract Number 9-8888, also in process of publication (1969)

6. Dietlein, L. F., and R. M. Rapp, Experiment M-3, Inflight Exercise Work Tolerance, Gemini Midprogram Conference Including Experiment Results, National Aeronautics and Space Administration, Manned Spacecraft Center, NASA SP-121:393 (1966)

7. Allison, Nathanial, and Barney Brooks, Surgery, Gynaecology, and Obstetrics, 33:250 (1921) quoted through Cuthbertson, David Paton, on The Influence of Prolonged Muscular Rest on Metabolism, Biochemical Journal 23:1328 (1929)

8. Allison, Nathanial, and Barney Brooks, Bone Atrophy: A Clinical Study of the Changes in Bone which Result from Non-Use, Archives of Surgery, Volume 5:499 (1922)

9. Cuthbertson, David Paton, Biochemical Laboratory, Royal Infirmary and Institute of Pathology, The Influence of Prolonged Muscular Rest on Metabolism, Biochemical Journal 23:1328 (1929)

10. Beigel (1955), through Cuthbertson, David Paton, on The Influence of Prolonged Muscular Rest on Metabolism, Biochemical Journal 23:1328 (1929)

11. Shaffer (1908), American Journal of Physiology 22:445, through David Paton Cuthbertson, Biochemical Journal 23:1328 (1929)

12. Albright, F., C. H. Burnett, O. Cope, and W. Parsons, Acute Atrophy of Bone Osteoporosis Simulating Hyperparathyroidism, Journal of Clinical Endocrinology 1:711 (1941)

13. Howard, John Eager, William Parson, and Roy S. Bingham, Studies on Patients Convalescing from Fracture: III. The Urinary Excretion of Calcium and Phosphorus, Johns Hopkins Hospital Bulletin, 77:291 (1945)

14. Taylor, Henry Longstreet, Lester Erickson, Austin Henschel, and Ancel Keys, The Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, The Effect of Bed Rest on the Blood Volume of Normal Young Men, American Journal of Physiology 144:227 (1945)

15. Taylor, Henry Longstreet, Austin Henschel, Josef Brozek, and Ancel Keys, Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Effects of Bed Rest on Cardiovascular Function and Work Performance, Journal of Applied Physiology 2:223 (1949)

16. Deitrick, John E., G. Donald Whedon, and Ephraim Shorr, The Effects of Immobilization Upon Various Metabolic and Physiologic Functions of Normal Men, American Journal of Medicine 4:3 (1948)

17. Whedon, G. Donald, John E. Deitrick, and Ephraim Shorr, Modification of the Effects of Immobilization upon Metabolic and Physiologic Functions of Normal Men by the Use of an Oscillating Bed, American Journal of Medicine 6:684 (1949)

18. Whedon, G. Donald, and Ephraim Shorr, Metabolic Studies in Paralytic Acute Anterior Poliomyelitis II. Alterations in Calcium and Phosphorus Metabolism, Journal of Clinical Investigation 73:966 (1957)

19. Lynch, Theodore N., Robert L. Jensen, Paul M. Stevens, Robert L. Johnson, and Lawrence E. Lamb, Metabolic Effects of Prolonged Bed Rest: Their Modification by Simulated Altitude, Aerospace Medicine, 38 (1): p. 10 (January, 1967)

20. Birkhead, N. C., J. J. Blizzard, J. W. Daly, G. J. Haupt, B. Issekutz, Jr., R. N. Myers, and K. Radahl, Cardiodynamic and Metabolic Effects of Prolonged Bed Rest, Technical Documentary Report No. AMRL-TDR-63-37, Biomedical Laboratory, Air Force System Command, Wright-Patterson Air Force Base, Ohio (1963)

21. Levine, Samuel A., Some Harmful Effects of Recumbency in the Treatment of Heart Disease, Journal of the American Medical Association, Volume 126, Number 2:80 (1944)

22. Eastman, Nicholson J., The Abuse of Rest in Obstetrics, The Journal of the American Medical Association, Volume 125, Number 16 (1944)

23. Oberfield, Richard A., Franklin G. Ebaugh, Jr., Edward P. O'Hanlon, and Melany Schoaf, Blood Volume Studies During and After Immobilization in Human Subjects as Measured by the Chromium^{51} Technique, Aerospace Medicine, Volume 39, Number 1:10 (1968)

24. Issekutz, B., Jr., J. J. Blizzard, N. C. Birkhead, and K. Rodahl, Effect of Prolonged Bed Rest on Urinary Calcium Output, Journal of Applied Physiology, Volume 21, Number 3:1013 (1966)

25. Heaney, Robert P., Radioactive Calcium Metabolism in Disuse Osteoporosis in Man, American Journal of Medicine, Volume 33:188 (1962)

26. Davidson, Sir Stanley, A. P. Meiklejohn, and R. Passmore, Human Nutrition and Dietetics, Third Edition, Part III, Chapter 29: 396, Williams and Wilkins Company, Publishers, Baltimore (1966)

27. Lutwak, Leo, and G. Donald Whedon, Osteoporosis-- A Disorder of Mineral Nutrition, Borden's Review of Nutrition Research, Volume 23, Number 4:45 (1962)

28. Kottke, Frederic J., The Effects of Limitation of Activity upon the Human Body, Journal of the American Medical Association, Volume 196, Number 10:117-122 (1966)

29. Kosmolinsky, Fyodor, and Boris Dushkov, Specific Features of Adaptation of a Human Organism to Prolonged Stay in Sealed Chambers, Aerospace Medicine, Volume 39, Number 5:508 (1968)

30. Van Liere, Edward J., Helen H. Hess, and James E. Edwards, Effect of Physical Training on the Propulsive Motility of the Small Intestine, Journal of Applied Physiology, Volume 7, Number 2: 186 (1954)

31. Chapman, A. L., Interview on: The Latest on Exercise and What it Can Do for You, U. S. News and World Report, Volume 46, Number 23:104 (June 8, 1959)

32. Davis, Elwood Craig, Gene A. Logan, and Wayne C. McKinney, Biophysical Values of Muscular Activity, Second Edition, William C. Brown Company, Publisher, Dubuque, Iowa (1965)

33. Matthias, Eugen, The Deeper Meaning of Physical Education, A. S. Barnes and Company, Publishers, New York (1929)

34. Brannon, Earl W., Charles A. Rockwood, and Pauline Potts, The Influence of Specific Exercises in the Prevention of Debilitating Musculoskeletal Disorders; Implication in Psychological Conditioning for Prolonged Weightlessness, Aerospace Medicine, Volume 34, Number 9:900 (1963)

35. Elton, Richard C., Stress Reaction of Bone in Army Trainees, Journal of the American Medical Association, Volume 204, Number 4:104 (1968)

36. Ganslen, Richard V., The Influence of Training and Exercise upon the Physiological and Mechanical Properties of Muscle and Connective Tissue, Revista de Educacion Fisica (Tlaxti) Volume 4, Number 2:3 (1960)

37. Stenberg, Jesper, Per-Olof Åstrand, Björn Ekblom, Joseph Royce, and Bengt Saltin, Homodynamic Response to Work with Different Muscle Groups, Sitting and Supine, Journal of Applied Physiology, Volume 22, Number 1:61 (1967)

38. Bevegård, Sture, Ulla Freychuss, and Tore Strandell, Circulatory Adaptation to Arm and Leg Exercise in Supine and Sitting Positions, Journal of Applied Physiology, Volume 21, Number 1:37 (1966)

39. Cooper, Kenneth H., and John W. Ord, Physical Effects of Seated and Supine Exercise with and without Subatmospheric Pressure Applied to the Lower Body, Aerospace Medicine, Volume 39, Number 5:481 (1968)

40. Greenleaf, J. E., Elizabeth M. Prange, and E. G. Averkin, Physical Performance of Women following Heat-Exercise Hypohydration, Journal of Applied Physiology, Volume 22, Number 1:55 (1967)

41. Wasserman, Karlman, Antonius L. Van Kessel, and George G. Burton, Interaction of Physiological Mechanisms during Exercise, Journal of Applied Physiology, Volume 22, Number 1:71 (1967)

42. Raab, W., P. de Paula E. Silva, H. Marchet, E. Kimura, and Y. K. Starcheska, Cardiac Adrenergic Preponderance due to Lack of Physical Exercise and Its Pathogenic Implications, The American Journal of Cardiology, Volume 5:300 (1960)

43. Wisham, Lawrence H., Arthur S. Abramson, and Alfred Ebel, Value of Exercise in Peripheral Arterial Disease, Journal of the American Medical Association, Volume 153, Number 1:10 (1953)

44. Smith, J. Eldrid, and George J. Kidera, Effect of Programmed Exercise on Non-Specific T-Wave Abnormalities, Aerospace Medicine, Volume 39, Number 5:528 (1968)

45. Proceedings of an International Symposium on Physical Activity and Cardiovascular Health, Canadium Medical Association Journal, Volume 96, Number 12 (March 25, 1967)

46. Evans, F. Gaynor, Biomechanical Studies of the Musculo-Skeletal System, Charles C. Thomas, Publisher, Springfield, Illinois (1961)

47. USSR Ministry of Health, Report on Sixty-two Day Bed Rest, Translation Eighteenth Congress of the International Federation (Belgrade, September, 1967) Reported by the National Aeronautics and Space Administration - TT F-11:399 (December 1967)

48. McLean, Franklin C., and Marshall R. Urist, Bone, The University of Chicago Press, Publishers, Chicago (1968)

49. Hendricks, Sterling B., and William L. Hill, The Inorganic Constitution of Bone, Science, Volume 96, Number 2489:255 (1942)

50. Rasch, Philip J., and Roger K. Burke, Kinesiology and Applied Anatomy: The Science of Human Movement, Third Edition, Lea and Febinger, Philadelphia (1967)

51. Geiser, Max, and Joseph Trueta, Muscle Action, Bone Rarefaction and Bone Formation, The Journal of Bone and Joint Surgery, Volume 40 B, Number 2:282 (1958)

52. Lipschütz, Alexander, and Alexander Audova, The Comparative Atrophy of the Skeletal Muscle after Cutting the Nerve and after Cutting the Tendon, Journal of Physiology, Volume 55, Number 4:300 (1921)

53. Kharmosh, Oved, and Paul D. Saville, The Effect of Motor Denervation on Muscle and Bone in the Rabbit's Hind Limb, Acta Orthopedics Scandinavia, Volume 36, Number 4:361 (1965)

54. McArdle, William D., Metabolic Stress of Endurance Swimming in the Laboratory Rat, Journal of Applied Physiology, Volume 22, Number 1:50 (1967)

55. Williams, Dorothy E., Rossie L. Mason, and Bonnie B. McDonald, Bone Density Measurements throughout the Life Cycle of the Rat, Journal of Nutrition, Volume 84, Number 4:373 (1964)

56. Williams, Dorothy E., Bonnie B. McDonald, Elise Morrell, Frances A. Schofield, and Florence L. McLeod, Influence of Mineral Intake on Bone Density in Humans and in Rats, The Journal of Nutrition, Volume 61, Number 4:489 (1957)

57. Hatch, Anita, Tibor Balazs, G. S. Wiberg, and H. C. Grice, Long Term Isolation Stress in Rats, Science, Volume 142, Number 3591: 507 (1963)

58. Slack, H. G. B., Metabolism of Limb Atrophy in the Rat, Clinical Science, Volume 13, Number 1:155 (1954)

59. Mueller, Werner J., Rosemary Schraer, and Harold Schaer, Calcium Metabolism and Skeletal Dynamics of Laying Pullets, The Journal of Nutrition, Volume 84, Number 1 (1964)

60. Crandall, L. A., Effect of Physical Exercise on the Gastric Secretion, American Journal of Physiology, Volume 84, Number 1:48 (1928)

61. Mack, Pauline Beery, Rudolf A. Hoffman, Aliya Al-Shawi, Physiologic and Metabolic Changes in Macaca Nemistrina on Two Types of Diets during Restraint and Non-Restraint: II. Bone Density Changes, Aerospace Medicine, Volume 39, Number 7:698 (1968)

62. Gross, Arthur L., Kenneth T. Roberson, Louis H. Krough, Jr., A Study of Calcium, Phosphorus, and Nitrogen Mobilization Resulting from Conditions of Inactivity in Macaca Irus Monkeys, Contract Air Force 41(609)2749, U. S. School of Aerospace Medicine, Aerospace Medical Division (AFSC), Brooks Air Force Bases, Texas

63. Kaplan, Harold P., Anthony A. Thomas, Kenneth C. Back, and Ferrel R. Robinson, Evaluation of Animals Exposed to a 5 p512 Pure Oxygen Space Cabin Atmosphere for Eight Months, Aerospace Medicine, Volume 39, Number 1:63 (1968)

64. Akeson, Wayne H., and Duane F. La Violette, The Connective Tissue Response to Immobility: Total Muscopolysaccharide Changes in Dog Tendon, Journal of Surgical Research, Volume 4, Number 11: 523 (1964)

65. Chor, H., and R. D. Dokart, A Study of "Simple Disuse Atrophy" in the Monkey, American Journal of Physiology, Volume 117, Number 4 (1936)

66. Harrison, M., and Russell Fraser, Bone Structure and Metabolism in Calcium-Deficient Rats, Journal of Endinchronology, Volume 21, Number 2:197 (1960)

67. Mack, Pauline Beery, Automated Techniques in Bone Densitometry, Journal of the Association for the Advancement of Medical Instrumentation, Volume 3, Number 3:110 (1969)

68. Mack, Pauline Beery, and Janice M. Smith, Methods of Conducting Mass Studies in Human Nutrition, The Pennsylvania State University Bulletin, Volume 33, Number 43:51 (1938)

69. Mack, Pauline Beery, Anne T. O'Brien, Janice M. Smith, and Arthur W. Bauman, A Method for Estimating the Degree of Mineralization of Bones from Tracing of Roentgenograms, Science, Volume 89, Number 2316:467 (1939)

70. Lowther, Mary E., Pauline Beery Mack, Catherine H. Logan, Anne T. O'Brien, Janice M. Smith, and Phyllis K. Sprague, The School Lunch as a Supplement to the Home Diet of Grade School Children, Child Development, Volume 11, Number 3:203 (1940)

71. Zayaz, Stella L., Pauline Beery Mack, Phyllis K. Sprague, and Arthur W. Bauman, Nutritional Status of School Children in a Small Industrial City, Child Development, Volume 11, Number 1:1 (1940)

72. Mack, Pauline Beery, Janice M. Smith, Catherine H. Logan, and Anne T. O'Brien, Nutritional Status of Children in a College Community, Journal of the American Dietetic Association, Volume 18, Number 1:69 (1942)

73. Mack, Pauline Beery, and C. Urbach, A Contribution to the Study of Rural and Urban Populations: I. General Physical Status and Skeletal Mineralization of Rural and Urban Non-Metropolitan Subjects. II. Preliminary report on Physical Status, Biochemical Findings, and Skeletal Maturation and Mineralization of Metropolitan School Children, The Pennsylvania State University Bulletin, Volume 36, Number 52 (1942)

74. Brown, Walter N., Jr., Semi-automatic Bone Density Computing Machine, Report to the National Electronics Conference (1949)

75. Mack, Pauline Beery, Results from the Study of Bone Density in the Appraisal of Calcium Status, Papers presented at a round table on Nutrition in Relation to Health and Disease at the 1949 Conference of the Milbank Memorial Fund. Published by the Milbank Memorial Fund, p. 30 (November 16-17, 1949)

76. Mack, Pauline Beery, Radiographic Bone Densitometry, Proceedings of Conference under sponsorship of the National Aeronautics and Space Administration, the National Institutes of Health, and the American Institute of Biological Sciences, NASA SP-64, p. 31 (March 25-27, 1965)

77. Mack, Pauline Beery, Walter N. Brown, Jr., and Hughes Daniel Trapp, Quantitative Evaluation of Bone Density, American Journal of Roentgenology and Radium Therapy, 61, 808 (1949)

78. Mack, Pauline Beery, George P. Vose, and James Donald Nelson, New Development in Equipment for Roentgenographic Measurement of Bone Density, American Journal of Roentgenology, Radium Therapy and Nuclear Medicine, Volume 82, Number 2:303 (1959)

79. Nelson, James Donald, Pauline Beery Mack, and George P. Vose, New Design of a Linearizing Recording Densitometer, Review of Scientific Instruments, Volume 29, Number 4:316 (1958)

80. Vogt, Fred B., Louis S. Meharg, and Pauline Beery Mack, Use of a Digital Computer in the Measurement of Roentgenographic Bone Density, The American Journal of Roentgenology, Radium Therapy and Nuclear Medicine, Volume 105, Number 4:870 (1969)

81. Mack, Pauline Beery, George P. Vose, Fred B. Vogt, and Paul A. LaChance, Experiment M-6 on Bone Demineralization, Manned Spaceflight Experiments. Proceedings of Symposium on Gemini Missions III and IV, National Aeronautics and Space Administration, Washington, D.C., p. 61 (October 19, 1965)

82. Mack, Pauline Beery, George P. Vose, Fred B. Vogt, and Paul A. LaChance, Experiment M-6 on Bone Demineralization, Proceedings of Manned Spacecraft Center Experiments Interim Report, Gemini V Mission, National Aeronautics and Space Administration, Washington, D.C., p. 109 (January 6, 1966)

83. Mack, Pauline Beery, George P. Vose, Fred B. Vogt, and Paul A. LaChance, Experiment M-6 on Bone Demineralization. Gemini VII Mission, Proceedings of Gemini Midprogram Conference, National Aeronautics and Space Administration, Manned Spacecraft Center, Houston, Texas, p. 407 (February 23-25, 1966)

84. Van Zandt, Dorothy Perkins, Circadian Rhythms of Urinary Calcium and Phosphorus in Healthy Adult Men during Recumbency and Ambulation, and the Effect of Exercise on These Excretions and Periodicities, Doctor of Philosophy Dissertation, Texas Woman's University (June, 1969)

85. Trueta, Joseph, Studies of the Development and Decay of the Human Frame, W. B. Saunders Company, Philadelphia (1968)

86. de Valderrama, Fernandez, and Joseph Trueta, The Effect of Muscle Action on the Intra-osseous Circulation, The Journal of Pathology and Bacteriology, Volume 89, Number 1:179 (1965)

87. Trueta, Joseph, The Dynamics of Bone Circulation, Conference on Bone Dynamics, Henry Ford Hospital, Detroit (1964)

88. Harris, William H., and Robert P. Heaney, Skeletal Renewal and Metabolic Bone Disease, New England Journal of Medicine, Volume 280, 193-202, 253-259,303-311, January 23, 30, February 6 (1969)

89. Mellanby, E., A Story of Nutritional Research: The Effect of Some Dietary Factors on Bone and the Nervous System, Williams and Wilkins, Publishers, Baltimore, Maryland (1950)

90. Mack, Pauline Beery, A Nutrition Study with College Girls Involving Four Levels of Fat Intake, Bulletin Number 2, Human Nutrition Series, Nelda Childers Stark Laboratory for Human Nutrition Research, p. 31 (1955)

91. Lutwak, Leo, Osteoporosis--A Mineral Deficiency, Journal of the American Dietetic Association, Volume 44, Number 3 (1964)

A P P E N D I X

Monthly Report 19 April, 1969

Under Contract NAS9-8246

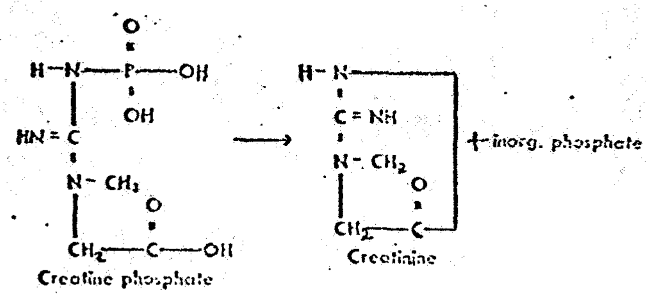
SUBJECT OF STUDY: Effects of Programmed Exercise on Skeletal Density and Calcium Metabolism during Horizontal Bed Rest of Healthy Adult Human Males

SUBJECT OF MONTHLY REPORT: EXCRETION OF CREATINE AND CREATININE DURING TWO 28-DAY BED REST PERIODS AND RELATED AMBULATORY PHASES OF A 113-DAY STUDY INVOLVING EXERCISE AT DIFFERENT LEVELS DURING HORIZONTAL BED REST

This study was conducted on six adult human males covering a span of 113 days, as has been described in previous monthly reports of this series. The subjects were observed during five different Periods— Pre-Bed Rest, Bed Rest I (no exercise), Interim Ambulatory, Bed Rest II (with exercise), and Post-Bed Rest Reconditioning. The study was designed mainly to determine the changes in bone density, as well as metabolism following oral administration of radioactive ^{47}Calcium, and the effects of exercise during bed rest on these factors.

FORMATION OF CREATININE

One of the most important end products of amino acid catabolism is creatinine which arises from creatine phosphate by ring-formation with the loss of phosphoric acid. The spontaneous decomposition of creatine phosphate to form creatinine can be shown by the following equation:



<u>Comparison of Two Methods of Analysis</u>

<u>for Creatine</u>

Two analytical methods were studied in connection with the analysis of creatine. One was the direct procedure in which creatine reacts with a chemical agent to produce a chromogen, and the other is the indirect procedure in which creatine is determined after dehydration to creatinine in acid media. The direct method for the analysis of creatine was developed by Raaflaub and Abelin (Raaflaub, J. and I. Abelin, <u>Biochemische Zeetschrift</u>, 321:158-165 (1950); and the indirect method by Folin (Folin, Otto, American Journal of Physiology, <u>13</u>:117-138 (1905)*. Both of these methods were evaluated in this study.

In order to determine the stability of the procedure for the direct method, the rate of color development was observed by following the rate of absorbency on the spectrophotometer. Readings were made after color development of 20 minutes, 30 minutes, 50 minutes and 70 minutes, plotted in Figure 1.

The analysis also was read at varying wave lengths from 450 mu to 540 mu in an effort to ascertain at which wave length the maximum

*as modified by Biggs and Cooper, Clinical Chemistry, 7:655 (1961) absorption is reached. The results are given in Figure 2 showing that peak absorption occurs between 520 mμ and 530 mμ.

A standard curve was prepared following this procedure. Five different standard concentrations containing 10, 20, 30, 40, and 50 μg. of creatine were used to determine the linear relationship. Figure 3 shows that this method follows Beer's Law.

The indirect modified Folin method had been used in this laboratory for a number of years in the analysis both of creatine and creatinine, because of the frequency of its use as reported in the literature. A comparative study of this method and the direct method of Raaflaub and Abelin in the analysis of creatine was made using urine containing known creatine amounts at different levels. With the latter method, 95 per cent recovery was found denoting a high degree of accuracy. Much lower levels of creatine were recovered in the modified Folin method of analysis. This report therefore is based on the results of the direct method for creatine, with creatinine analysis continuing according to the modified Folin method.

Creatinine has long been considered a normal constituent of the urine, being the second most abundant nitrogen containing compound in human urine. In the past, it was commonly believed that urinary creatinine was relatively constant, thereby providing a good indication as to the accuracy of a 24-hour urine collection. More recent investigations have dispelled this concept in favor of the theory that constancy is variable with the individual, and that creatinine excretion will be as variable as other urinary components. Various isotopic studies have confirmed the finding that 24-hour urinary creatinine output is insufficiently constant between individuals, or in the same individual from time to time, to justify its use as an accurate index of completeness of collections. Stress is one factor which may effect muscle contraction, thus causing increases in urinary creatinine.

Creatine can be detected in the urine of most normal subjects. Creatinuria is the condition in which creatine is found in the urine above that which is expected. Because of the variety of methods used for creatine determination and the variation in excretion among normal subjects, there is no sharp distinction as to the level at which creatinuria normally occurs.

Urinary Excretion of Creatinine

On careful examination of the statistical data relating to changes in the excretion of urinary creatinine, it is noted that all subjects exhibited a significant increase ($P<0.001$) during Bed Rest I and Bed Rest II, as compared with the Pre-Bed Rest Period. When the data were pooled for all six subjects, the amounts excreted were significantly higher during both bed rests.

No statistical difference was observed in any subject between the creatinine excretions reported for Bed Rest I while the subjects were not participating in exercise, as opposed to Bed Rest II in which the subjects were engaged in supervised exercise, although Bed Rest I was slightly higher in this respect for all subjects, as shown in Figure 4.

All subjects were found to show a significant difference when comparing the creatinine excretions of the Pre-Bed Rest Period with the Interim Ambulatory Period, with the Interim Period higher than the Pre-Bed Rest Period. No significant difference, however, was observed between the other ambulatory periods of the study.

With respect to the higher creatinine values observed in both bed rests, it may be that the element of stress involved in bed rest may be a factor in the elevated excretions. Previous studies have indicated stress to be a significant factor in increased amounts of urinary creatinine excretion.

Urinary Excretion of Creatine

Creatine excretion is shown to be significantly higher during both bed rest periods than during the Pre-Bed Rest Period in all subjects ($P<0.001$).

In comparing the results of the two bed rest periods, there was a significant difference in each subject between the bed rest periods in which the subjects exercised and the bed rest period during which the subjects did not participate in exercise, with Bed Rest I higher in each case ($P<0.001$). See Figure 5.

It also was noticed that, when Bed Rest I with no exercise was compared with the Post-Bed Rest Ambulatory Period, all subjects showed a statistically significant decrease ($P<0.001$) during the latter.

There was no significant difference demonstrated by either group when the Exercise Period was compared with the Interim Ambulatory Period.

When comparing the creatine values of the Exercise Period for the group exercising ad libitum with the Post-Bed Rest Period, no significant difference was noted. The values for the group who exercised regularly, however, showed a highly significant decrease in creatine excretion during the Post-Bed Rest Period as compared to the Exercise Period ($P<0.001$). In comparing the creatine excretions of the two exercise groups, it was shown that the subjects in the group exercising regularly in Bed Rest II had a lower mean urinary creatine excretion during Bed Rest I than did the ad libitum group. The difference between the two groups was highly significant ($P<0.001$). In comparing the creatine excretions of the two exercise groups during Bed Rest II in which both groups were participating in exercise to a different level, no significant difference was noted.

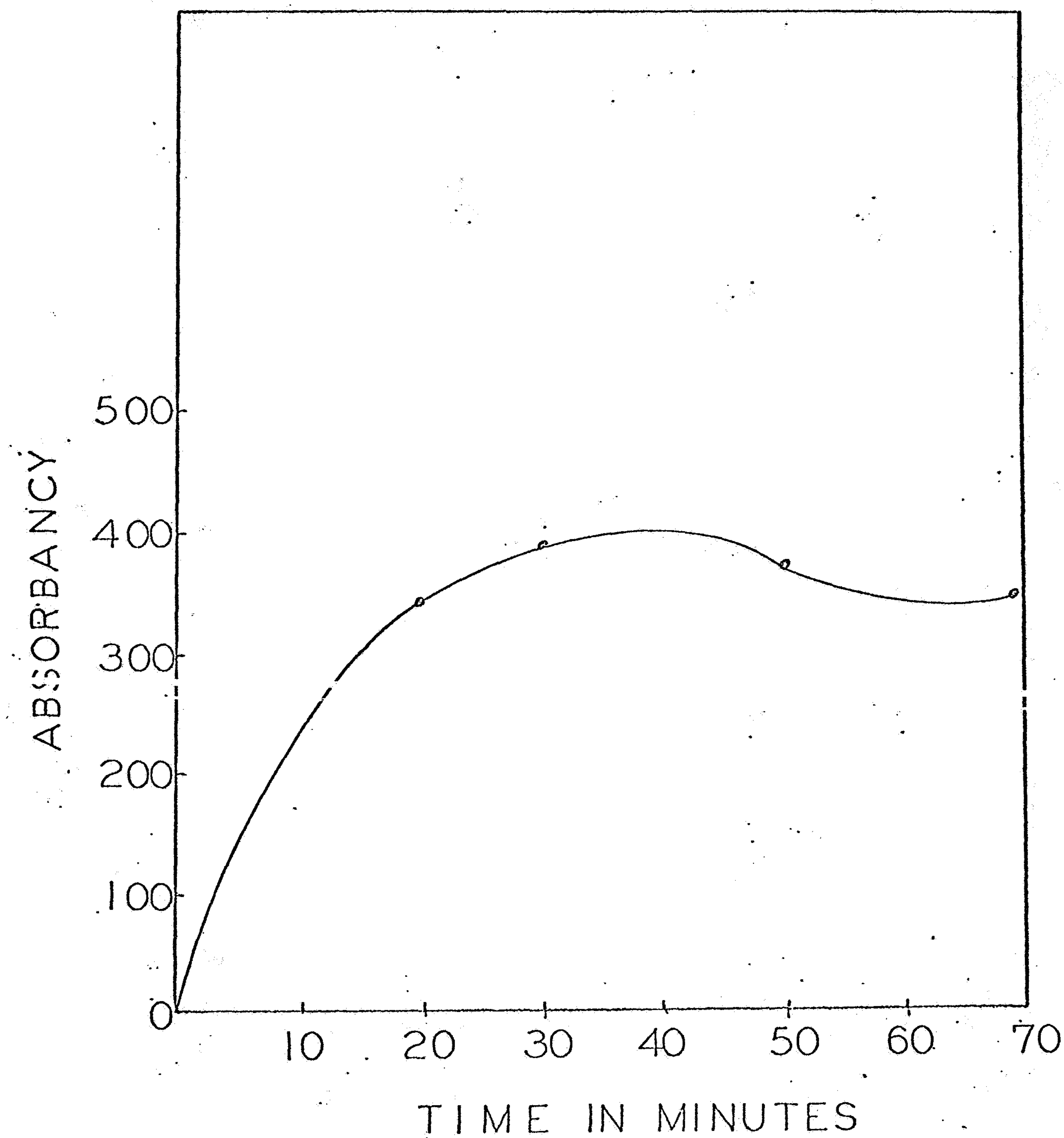


Figure 1. A TYPICAL CURVE SHOWING THE RATE AND STABILITY OF COLOR DEVELOPMENT FOR THE COLORIMETRIC DETERMINATION OF CREATINE BY THE METHOD OF RAAFLAUB AND ABELIN

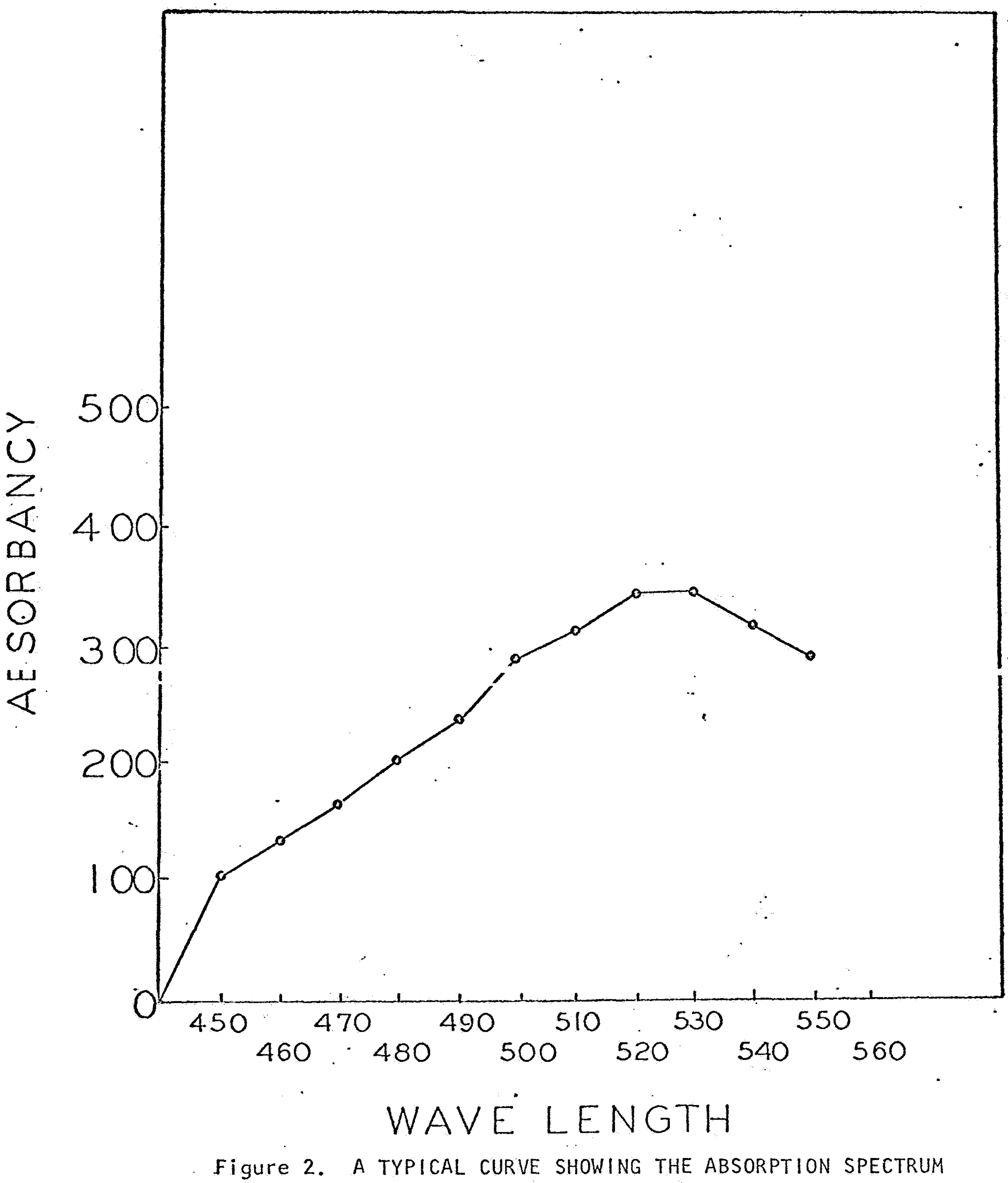


Figure 2. A TYPICAL CURVE SHOWING THE ABSORPTION SPECTRUM FOR THE COLORIMETRIC DETERMINATION OF CREATINE BY THE METHOD OF RAAFLAUB AND ABELIN

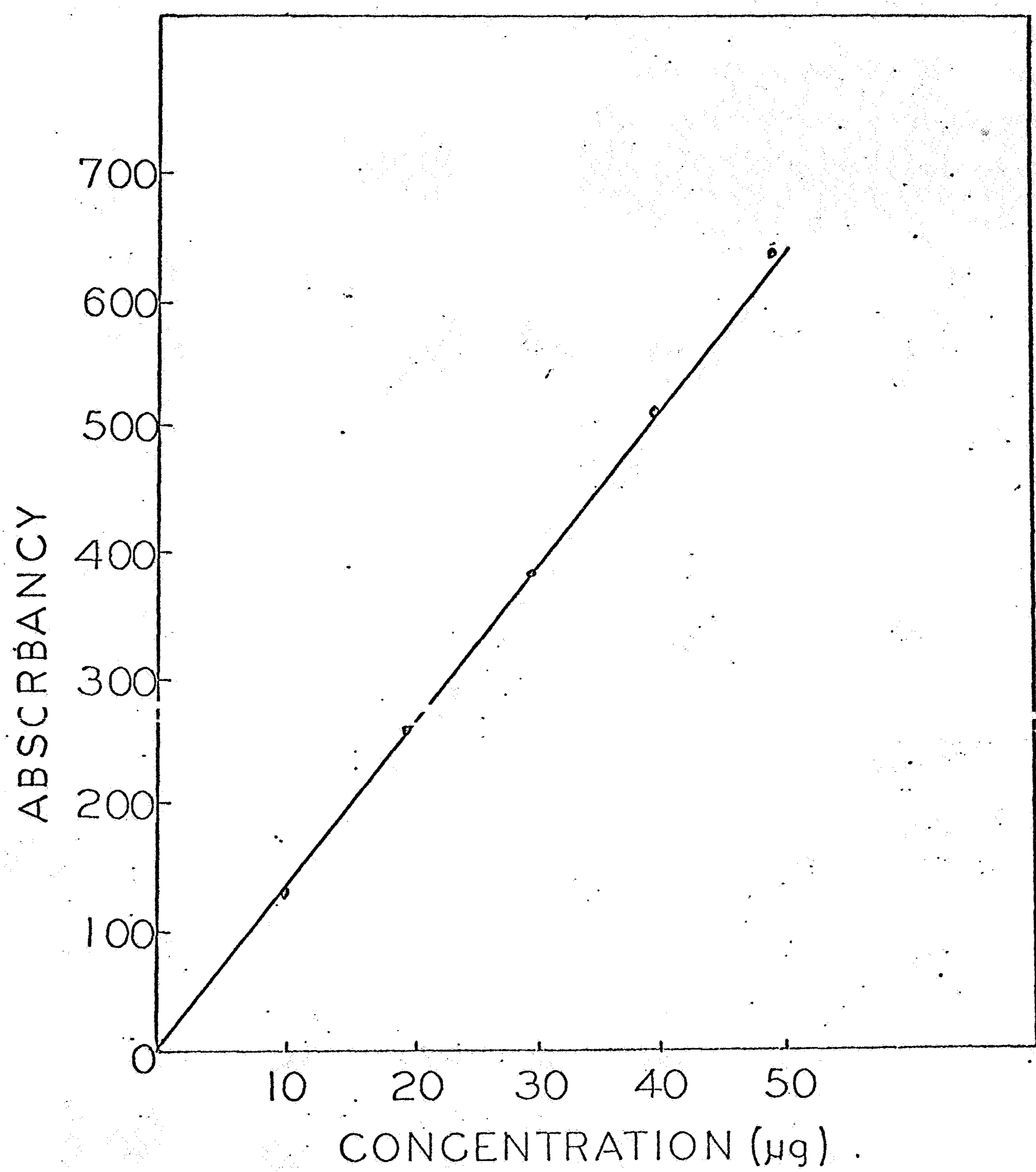


Figure 3. A TYPICAL STANDARD CURVE FOR THE COLORIMETRIC DETERMINATION OF CREATINE BY THE METHOD OF RAAFLAUB AND ABELIN

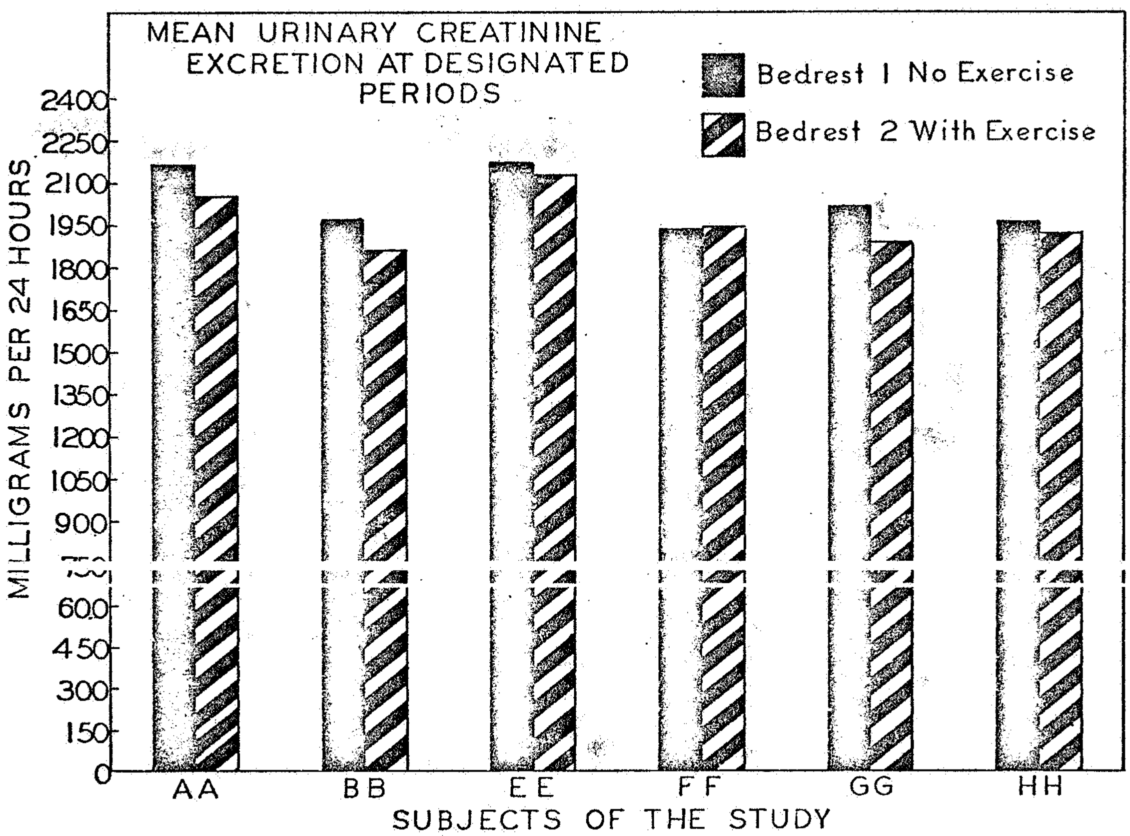


Figure 4. MEAN URINARY CREATININE EXCRETION DURING THE TWO BED REST PERIODS BY ALL SIX SUBJECTS IN THE STUDY

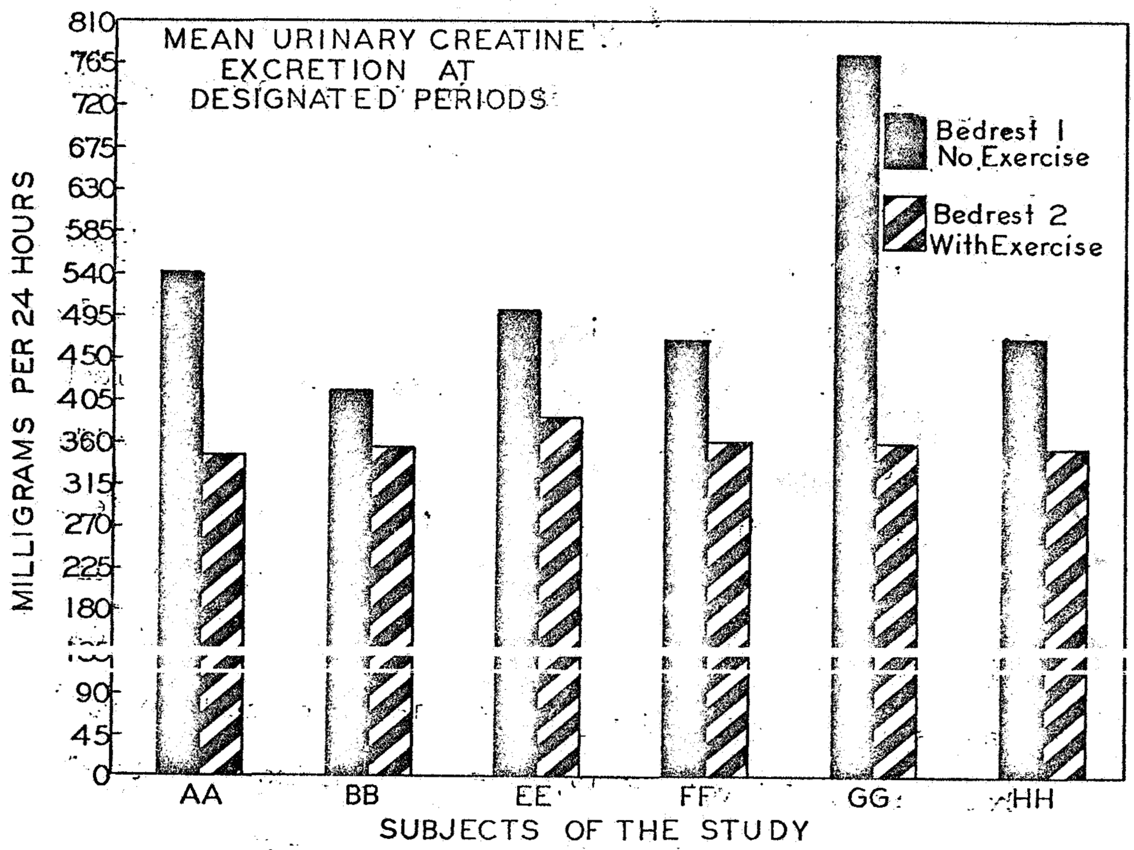


Figure 5. MEAN URINARY CREATINE EXCRETIONS DURING THE TWO BED REST PERIODS BY ALL SIX SUBJECTS IN THE STUDY

Monthly Report 19 May, 1969

Under Contract NAS9-8246

<u>SUBJECT OF STUDY</u>: Effects of Programmed Exercise on Skeletal Density and Calcium Metabolism during Horizontal Bed Rest of Healthy Adult Human Males

<u>SUBJECT OF MONTHLY REPORT</u>: CIRCADIAN RHYTHMS IN URINARY CALCIUM, PHOSPHORUS, NITROGEN, CREATINE, AND CREATININE

CIRCADIAN RHYTHM PHENOMENON

This report emphasizes the important and almost unexplored area of the Circadian Rhythm phenomenon. The evolutionary development of all living organisms, including man, took place under the influence of cosmic forces that have not changed appreciable for long periods of time. As a result, most physiological processes still are geared to these forces; and they exhibit cycles that have daily, seasonal, and other periodicities. While daily, lunar, seasonal, and other periodicities are a universal feature of biological phenomena, their precise mechanisms are still a matter of controversy. It has been stated that the most common assumption is that each organism possesses a number of "internal clocks" which indicate when a biological activity should begin.

For a long period of time, the constancy of the "milieu interieur" - now mostly referred to as homeostasis - has been well established. It has been believed that all vital mechanisms, however varied they might be, have but one goal, to maintain the uniformity of the conditions of life in the internal environment.

Of all biological rhythms, the ones with a daily periodicity have been most extensively studied. The term diurnal is still used in reference to daily rhythm; but since it has been shown that animals rarely exhibit a periodicity of exactly 24 hours, the term circadian (circa dies) has been adopted as more appropriate. Rhythm is influenced by environmental as well as physiologic factors.

Circadian vatiations of urinary excretions are among the problems most extensively studied, at present. It is difficult to achieve statistically valid variations in circulating blood, and hence numerous studies in this area have been made on urine. Urinary rhythms are among the easiest to studv under field conditions, since material can be collected for analysis after return to civilization.

The recumbency study of which this is a part was planned in such a way that circadian rhythms of various urinary components upon which analyses were made could be observed on Bed Rest I. During this period, the six subjects were in complete horizontal bed rest, with a constant diet and with no other variables which would interfere with the rhythmicity observations except during the first of the four weeks of this bed rest, when blood tests and other measurements were made which, it was believed, might interfere with this problem. During Bed Rest II, some observations also were made, although the variable of exercise was introduced, with three of the subjects exercising according to a schedule, and three "at will".

Circadian Rhythms of Urinary Calcium and Phosphorus

The pattern of a definite circadian rhythm was evident in the urinary calcium excretion during Bed Rest I, with the average daily minimum amount for each subject present in the urine sample accumulated at night, during the period ending a 8:00 A.M. The maximum amount of calcium was observed in the 8:00 A.M. to Noon collection. No diurnal periodicity was evident for phosphorus, however, during this Bed Rest. In some cases the phosphorus amounts during certain periods were opposite those of calcium. See Summaries A and B and Figures 1 and 2.

Figure 3 shows the circadian rhythms for urinary calcium during Bed Rest I for a representative subject for all days during three weeks when collections were finalized at standard times (8:00 A.M., 12 Noon, and 8:00 P.M.).

S U M M A R Y A

MEAN VALUES OF URINARY CALCIUM EXCRETION (IN MILLIGRAMS) DURING THE 28 DAYS OF BED REST I AT THE DESIGNATED PERIODS OF TIME

Subject	8 P.M.-8 A.M.	8 A.M.-12 Noon	12 Noon-8 P.M.
BB	11.6	22.5	13.5
FF	10.3	22.0	12.4
HH	9.8	17.2	11.8
GG	10.2	20.8	15.4
AA	15.4	30.0	14.8
EE	9.2	44.4	18.3

S U M M A R Y B

MEAN VALUES OF URINARY PHOSPHORUS EXCRETION (IN MILLIGRAMS) DURING THE 28 DAYS OF BED REST I AT THE DESIGNATED PERIODS OF TIME

Subject	8 P.M.-8 A.M.	8 A.M.-Noon	12 Noon-8 P.M.
BB	42.1	51.7	41.3
FF	40.5	29.3	43.9
HH	47.9	39.0	45.7
GG	45.0	45.1	46.7
AA	44.1	33.8	47.1
EE	28.3	83.0	54.7

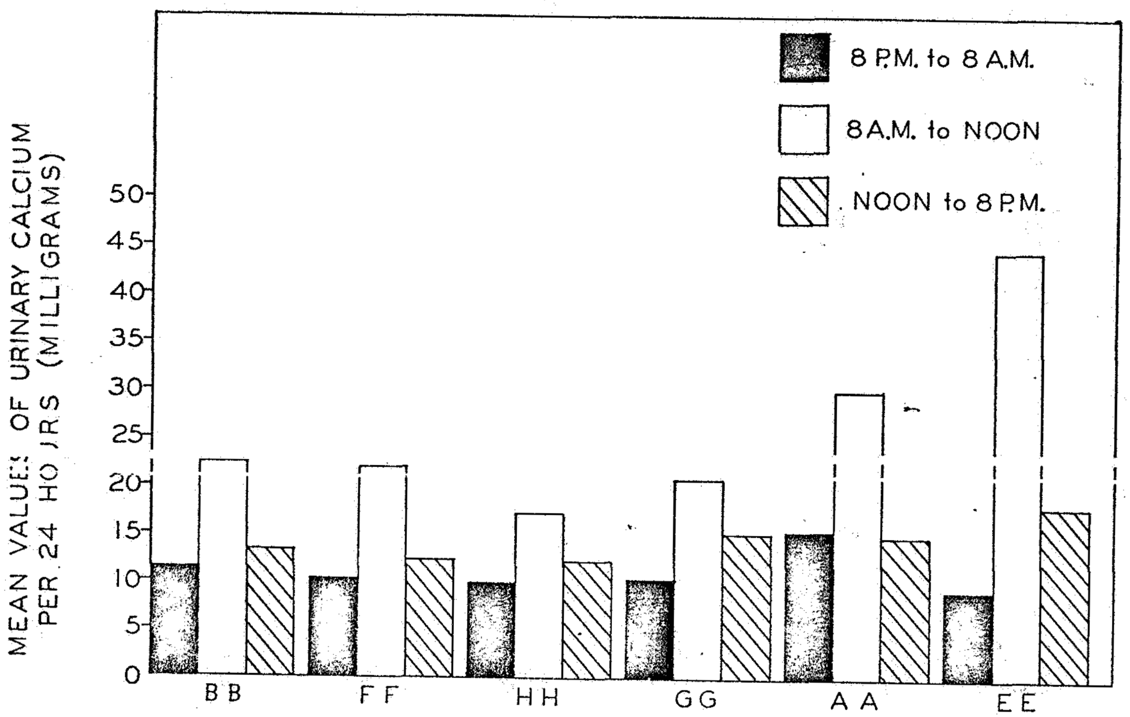


Figure 1. THESE BAR GRAPHS SHOW THE TOTAL MEAN URINARY CALCIUM EXCRETED DURING A 24-HOUR PERIOD, DIVIDED INTO THE THREE PORTIONS ELIMINATED FURING THE SUB-PERIODS DESIGNATED, FOR THE SIX SUBJECTS OF THE STUDY (BB, FF, HH, GG, AA, EE)

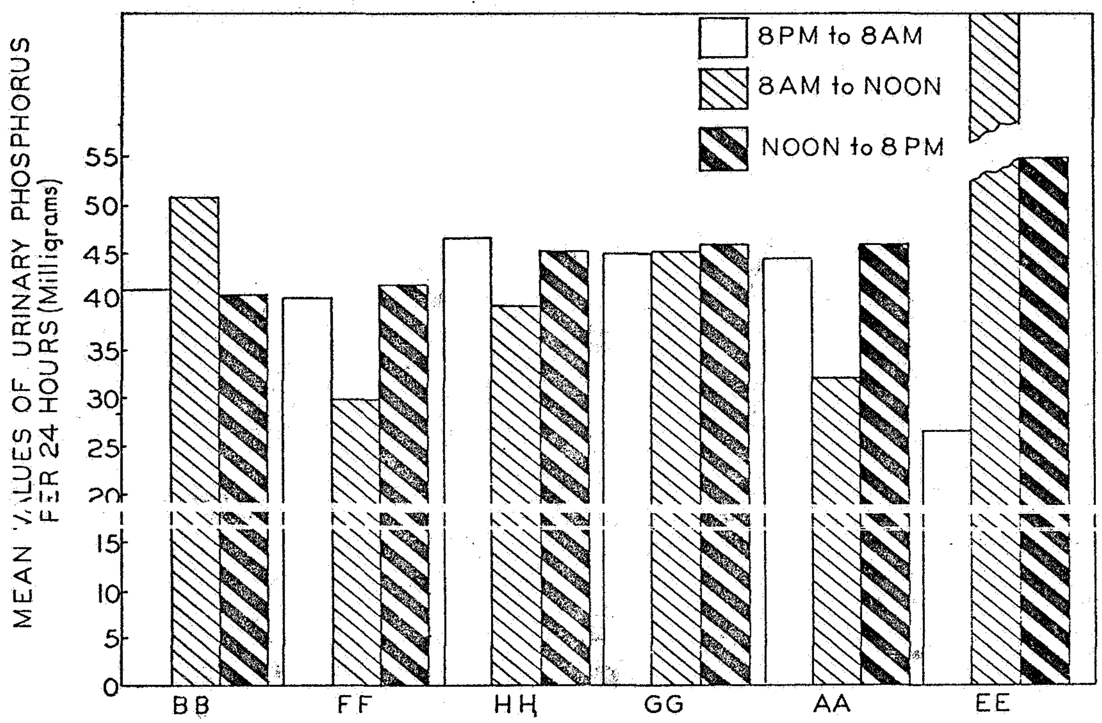
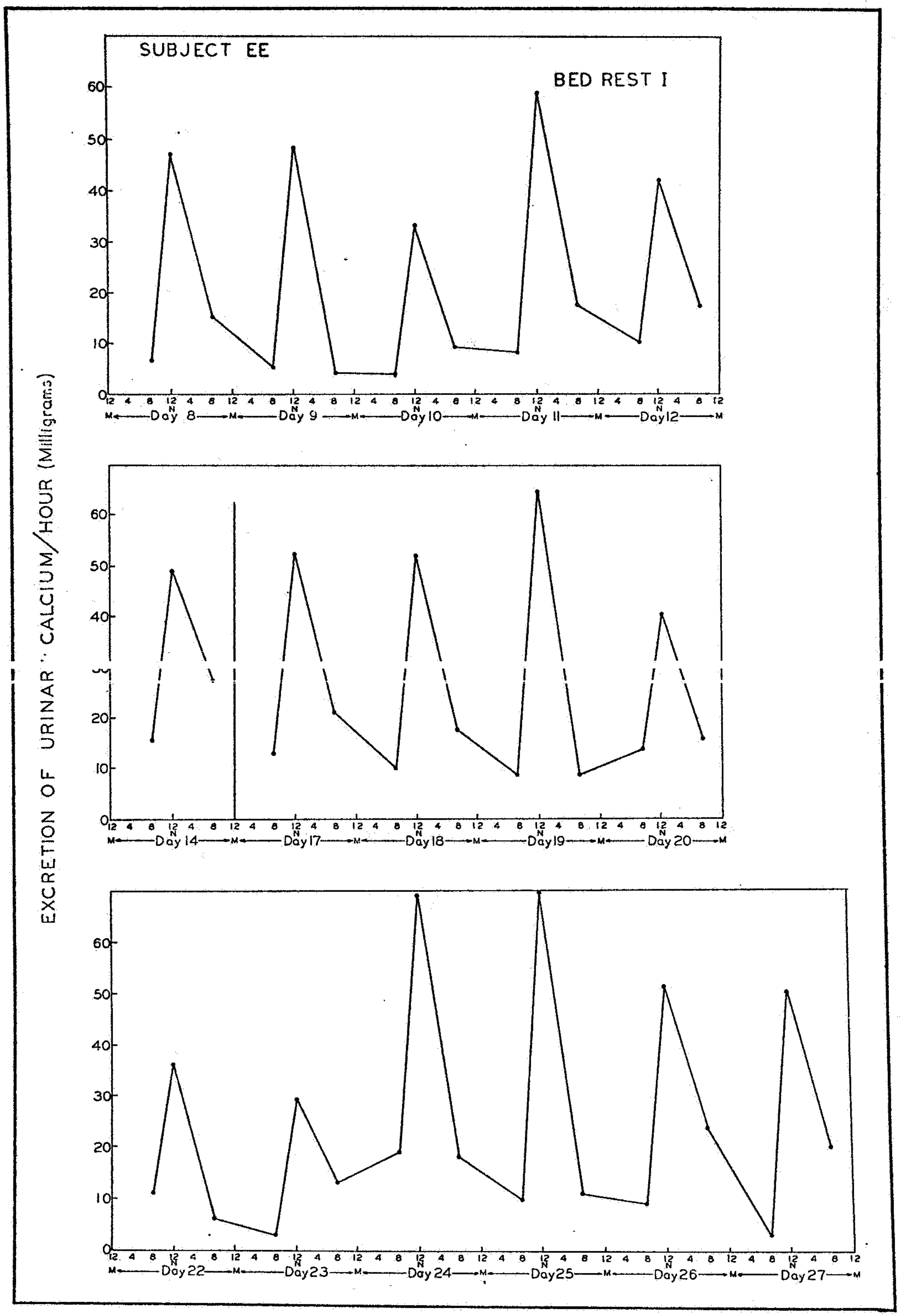


Figure 2. THESE BAR GRAPHS SHOW THE TOTAL MEAN URINARY PHOSPHORUS EXCRETED DURING A 24-HOUR PERIOD, DIVIDED INTO THE THREE PORTIONS ELIMINATED DURING THE SUB-PERIODS DESIGNATED, FOR THE SIX SUBJECTS OF THE STUDY (BB, FF, HH, GG, AA, EE)

Figure 3. URINARY CALCIUM CIRCADIAN RHYTHMS DEMONSTRATED DURING BED REST I (COMPLETE RECUMBENCY) FOR SUBJECT FF


SUBJECT EE
BED REST I
EXCRETION OF URINARY CALCIUM/HOUR (Milligrams)
Day 8
Day 9
Day 10
Day 11
Day 12
Day 14
Day 17
Day 18
Day 19
Day 20
Day 22
Day 23
Day 24
Day 25
Day 26
Day 27

Five of the six subjects lost less calcium during Bed Rest II, with exercise, than during Bed Rest I, without exercise. The one subject who excreted more calcium during the latter study was the one who exercised least. The highest excretion of urinary calcium for all subjects occurred during the period which ended at Noon.

Exercise appeared to alter the circadian rhythm program for calcium to some extent. The samples were not taken at the regular periods used in Bed Rest I because chemists at NASA Manned Spacecraft Center wanted aliquots of the urinary excretions for separate analyses collected whenever voiding occurred.

For those subjects exercising regularly during Bed Rest II, the lowest concentration of urinary calcium was present most often in the 8:00 P.M. to 8:00 A.M. collections, while the highest was noted most frequently in the 8:00 A.M. to 12 Noon samples. There then was usually a gradual decrease, sometimes followed by another peak in the late evening.

The subjects exercising "at will" excreted more calcium in the urine during Bed Rest II than did those exercising regularly, with the greatest amount observed from 9:00 A.M. to Noon, and the next largest quantity found from 3:00 P.M. to 6:00 P.M. These time periods of maximum excretion differed from those of the subjects exercising regularly.

The subjects exercising regularly exhibited the highest urinary phosphorus excretion during the 9:00 P.M. to the 12:00 Midnight period, and the lowest quantity from 6:01 A.M. to 9:00 A.M. Those subjects who exercised "at will" showed a somewhat different rhythmicity. Peak urinary phosphorus excretion for this group occurred from 6:01 P.M. to 9:00 P.M., as compared to 9:01 P.M. to 12:00 Midnight in the regularly exercising group. The least phosphorus excretion for the "at will" exercise group was noted from Noon to 3:00 P.M., in contrast to the period from 6:01 A.M. to 9:00 A.M. in the group exercising regularly.

Circadian Rhythms of Urinary Nitrogen

Urinary nitrogen followed the same general rhythm pattern during Bed Rest I as calcium, with the excretions from 8:00 A.M. until 12:00 Noon highest in this element, and the night excretions (8:00 P.M. until 8:00 A.M.) lowest. See Figure 4.

The nitrogen excretions during Bed Rest II when exercise was followed to different levels did not follow a regular pattern.

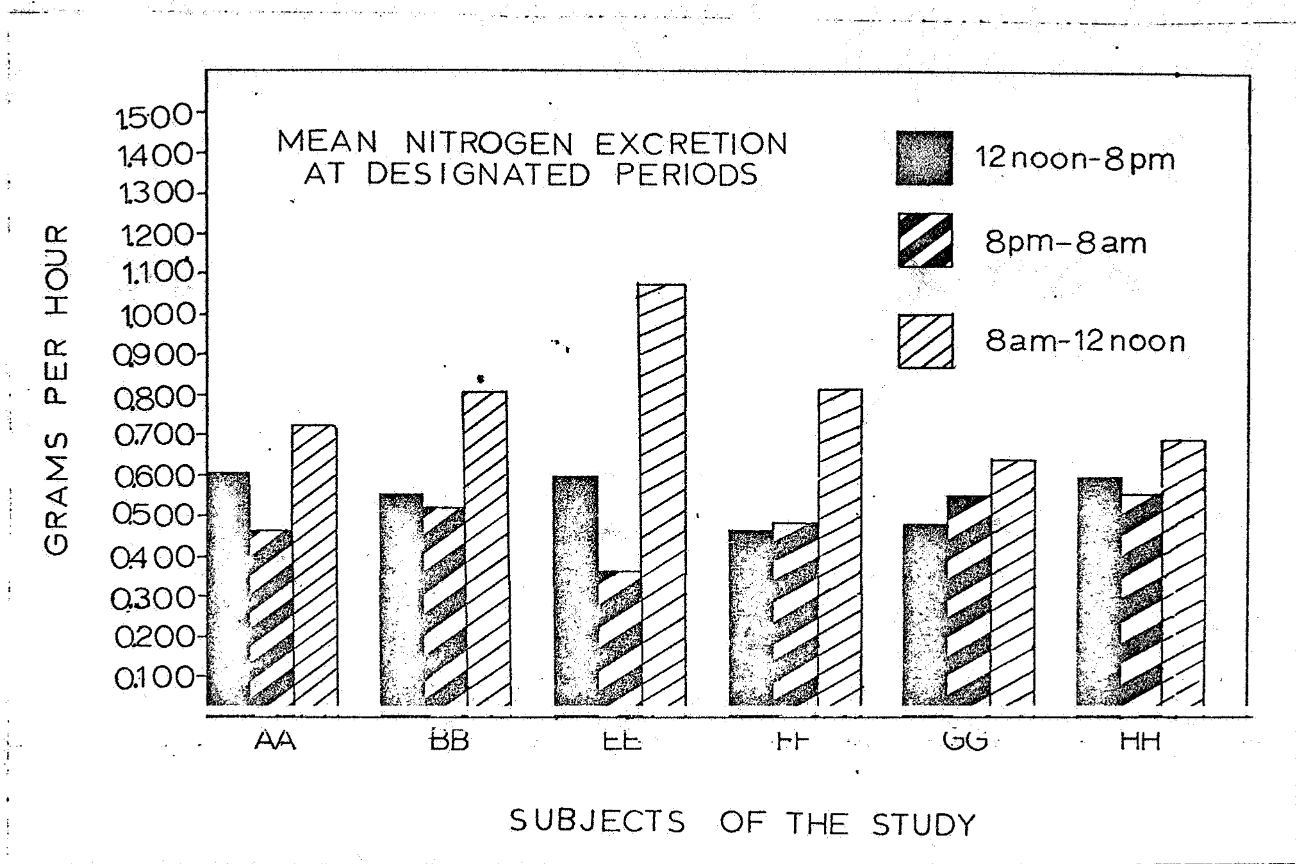


Figure 4. THESE BAR GRAPHS SHOW THE TOTAL MEAN URINARY NITROGEN EXCRETED IN BED REST I DURING A 24-HOUR PERIOD, DIVIDED INTO THE THREE PORTIONS ELIMINATED DURING THE SUB-PERIODS DESIGNATED, FOR THE SIX SUBJECTS OF THE STUDY (BB, FF, HH, GG, AA, EE)

CIRCADIAN RHYTHMS OF CREATININE AND CREATINE

Creatinine (No Exercise). The urinary creatinine excretion of all subjects during both bed rests was noted to be significantly higher ($P<0.001$) than during the Pre-Bed Rest Period. The excretion of creatinine was higher for all subjects during the period from 8:00 A.M. - 12 Noon than for the other collection periods. This increase showed a statistically significant difference in all cases, with a markedly significant difference when the data for all subjects were pooled ($P<0.001$).

The excretion rates during the periods from 12 Noon to 8 P.M. and from 8 P.M. to 8 A.M. were similar for all subjects, with all subjects exhibiting a slightly lower creatinine excretion during the overnight period (8 P.M. to 8 A.M.). Statistically this change was not significant, but it may be of some slight physiological significance. See Figure 5.

Creatine (No Exercise). The average urinary creatine excretion for all subjects was highest during the period from 8 A.M. to 12 Noon. In five of the subjects this increase was noted to be of statistical significance when compared to the other two collection periods. Although Subject HH showed an increase in creatine excretion during the 8 A.M. to 12 Noon period, it was not found to be statistically different from the other two periods.

Most of the subjects exhibited no significant change when the data for the period from 12 Noon to 8 P.M. was related to the period from 8 P.M. to 8 A.M. Subject EE, however, was noted to have a highly significant increase ($P<0.001$) in creatine excretion between the periods of

12 Noon to 8 P.M. as compared to 8 P.M. to 8 A.M.

It was of note that the creatine excretion was found to be lowest during the overnight period (8 P.M. to 8 A.M.). This exhibited no statistically significant difference when compared to the period 12 Noon to 8 P.M., but it may have some physiological importance.

See Figures 5, 6, and 7.

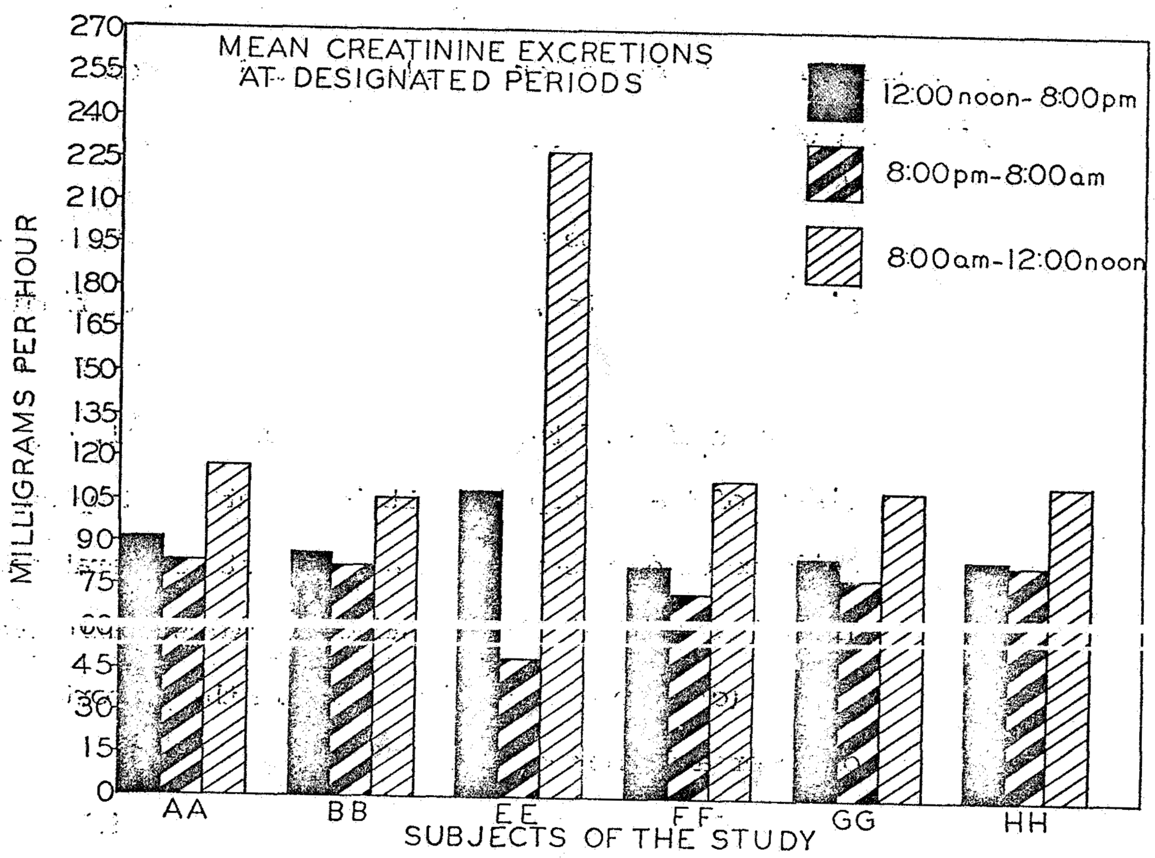


Figure 5. COMPARISON OF MEAN CREATININE EXCRETIONS DURING BED REST I (NO EXERCISE) AT DESIGNATED PERIODS OF COLLECTION

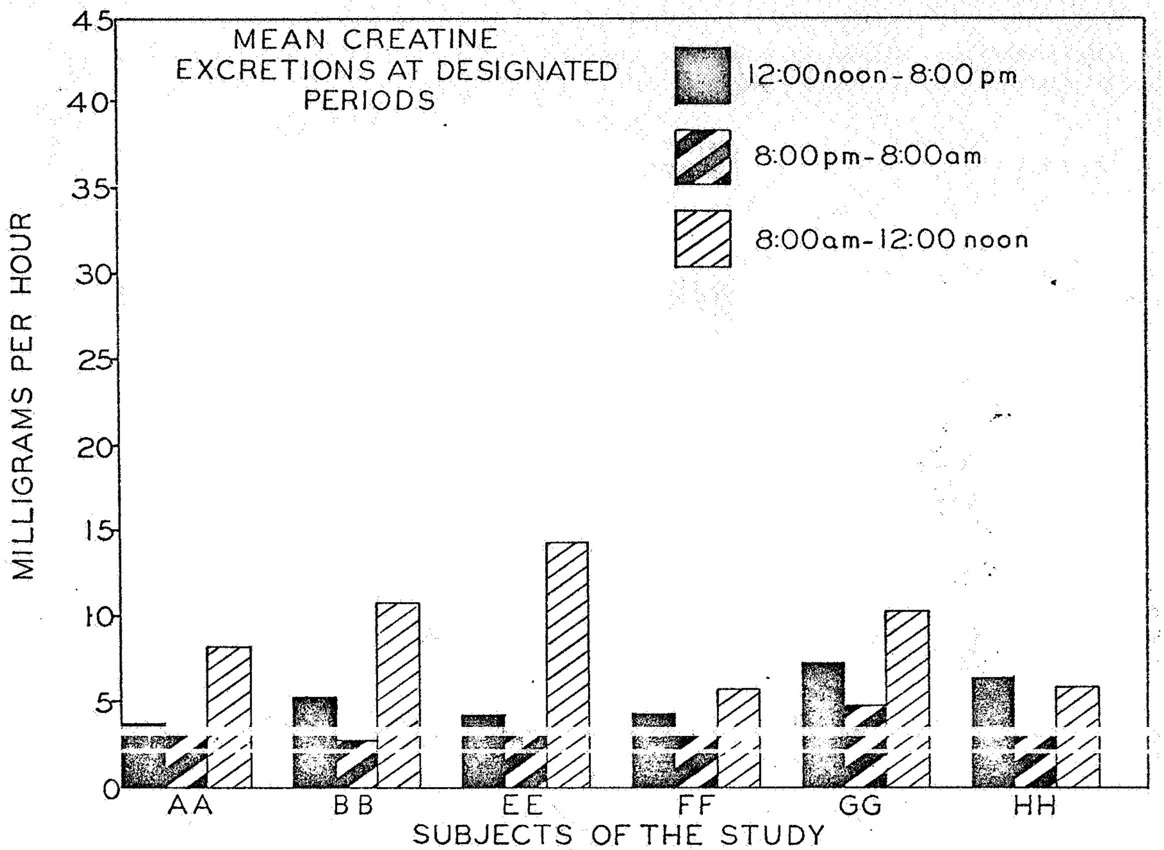


Figure 6. COMPARISON OF MEAN CREATINE EXCRETIONS DURING BED REST II (NO EXERCISE) AT DESIGNATED PERIODS OF COLLECTION USING THE ANALYTICAL PROCEDURE OF FOLIN AS MODIFIED BY BIGGS AND COOPER

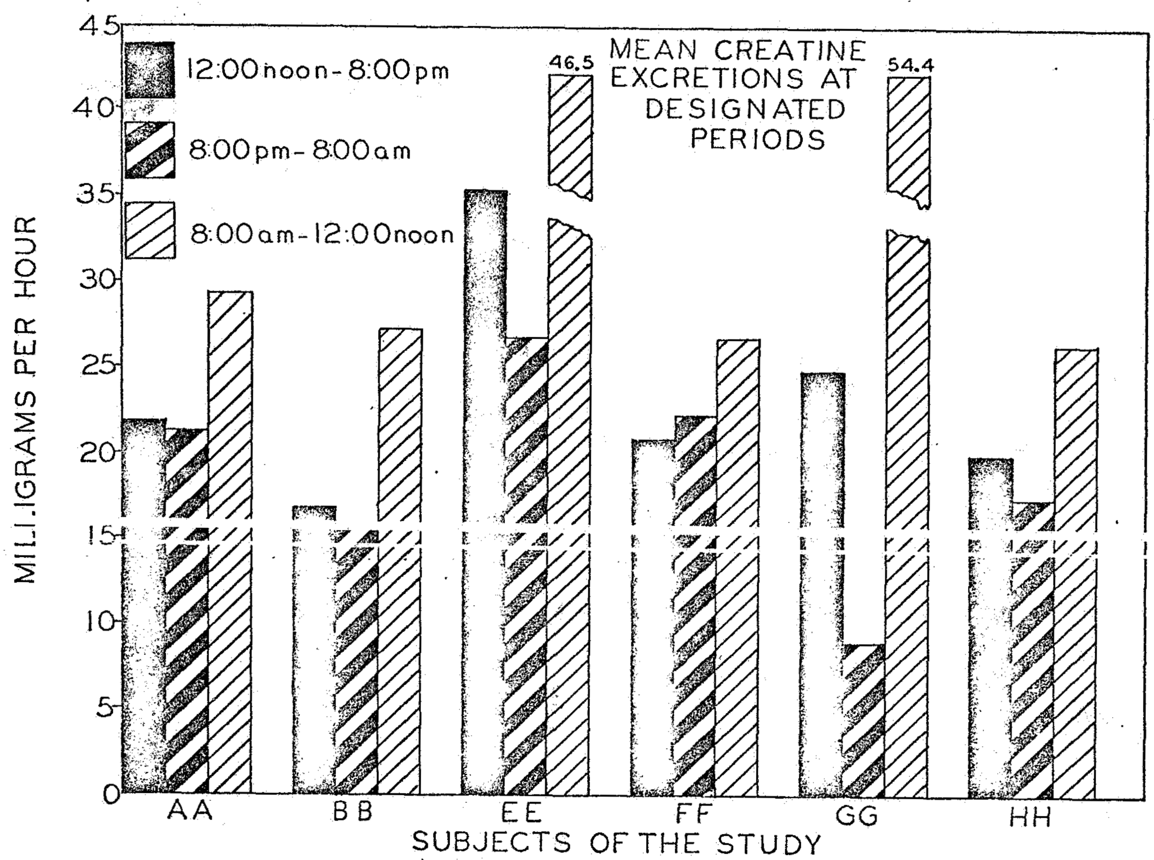


Figure 7. COMPARISON OF MEAN CREATINE EXCRETIONS DURING BED REST I (NO EXERCISE) AT DESIGNATED PERIODS OF COLLECTION USING THE ANALYTICAL PROCEDURE OF RAAFLAUB AND ABELIN

<u>Creatinine</u> (<u>With Exercise</u>). During Bed Rest II the subjects were divided into two groups-- one group of three subjects (AA, EE, GG) who exercised "at will" and one group of subjects (BB, FF, HH) who exercised on a regularly supervised schedule. During this period the individually collected urine samples for each subject were pooled and analyzed on a 24-hour basis for urinary creatine and creatinine.

In analyzing the data for the exercise period, both groups of subjects exhibited a highly significant increase ($P<0.001$) in urinary creatinine excretion during Bed Rest II when exercise was practiced as compared to Pre-Bed Rest I with no exercise. There was also a significant difference demonstrated by both groups of subjects when the creatinine excretions of the exercise period (Bed Rest II) were compared with the excretions of the Interim Ambulatory Period.

The group which exercised regularly showed no significant difference in creatinine excretions between the two bed rest periods while the group exercising "at will" demonstrated a slight degree of statistical difference between these periods. In both groups, however, the mean creatinine excretion was slightly higher during Bed Rest I when the subjects were not engaged in an exercise program.

When these two groups were compared as to their creatinine excretion for the exercise period itself, a highly significant change ($P<0.001$) was observed. Those subjects exercising ad libitum had a higher mean urinary creatinine excretion than those who exercised regularly.

<u>Creatine</u> (<u>With Exercise</u>). Both groups excreted a higher amount of urinary creatine during the Exercise Period than during the Pre-Bed Rest Period, with the difference highly significant. Both groups also excreted a higher amount of urinary creatine during Bed Rest I with no exercise, than during Bed Rest II, with exercise, with the difference also highly significant ($P<0.001$).

See Figures 8 and 9.

In comparing the creatine excretions of the two exercise groups (those who exercised regularly and those who exercised "at will". during Bed Rest II in which both groups were participating in exercise to a certain level, no significant difference was noted.

See Figure 10 for illustration of a Bed Rest subject exercising with an Exer-Genie while completely recumbent. Pull on the muscles related to the feet and legs assists in circulation and aids in maintaining the integrity of the skeleton provided the diet has supplied the blood with the nutrients needed by bones.

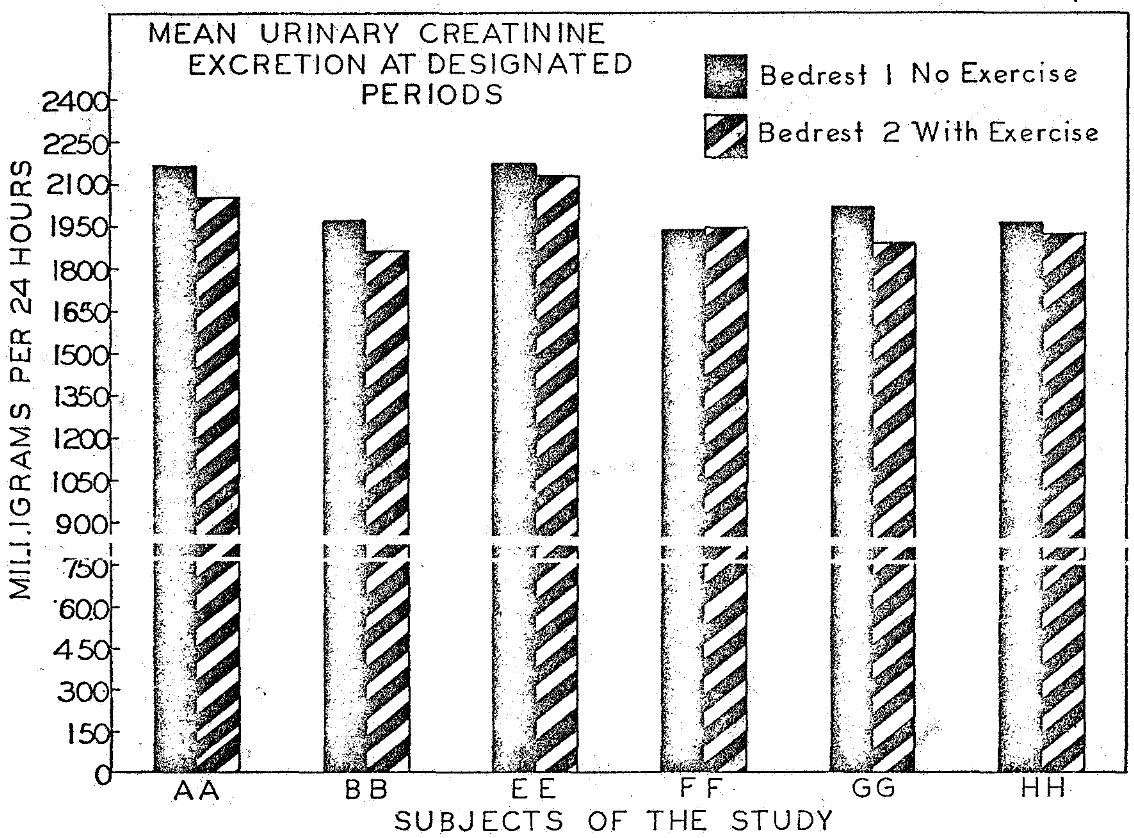


Figure 8. COMPARISON OF URINARY EXCRETION OF CREATININE DURING BED REST I (NO EXERCISE) AND BED REST II, WITH VARYING DEGREES OF EXERCISE FOR THE DIFFERENT SUBJECTS

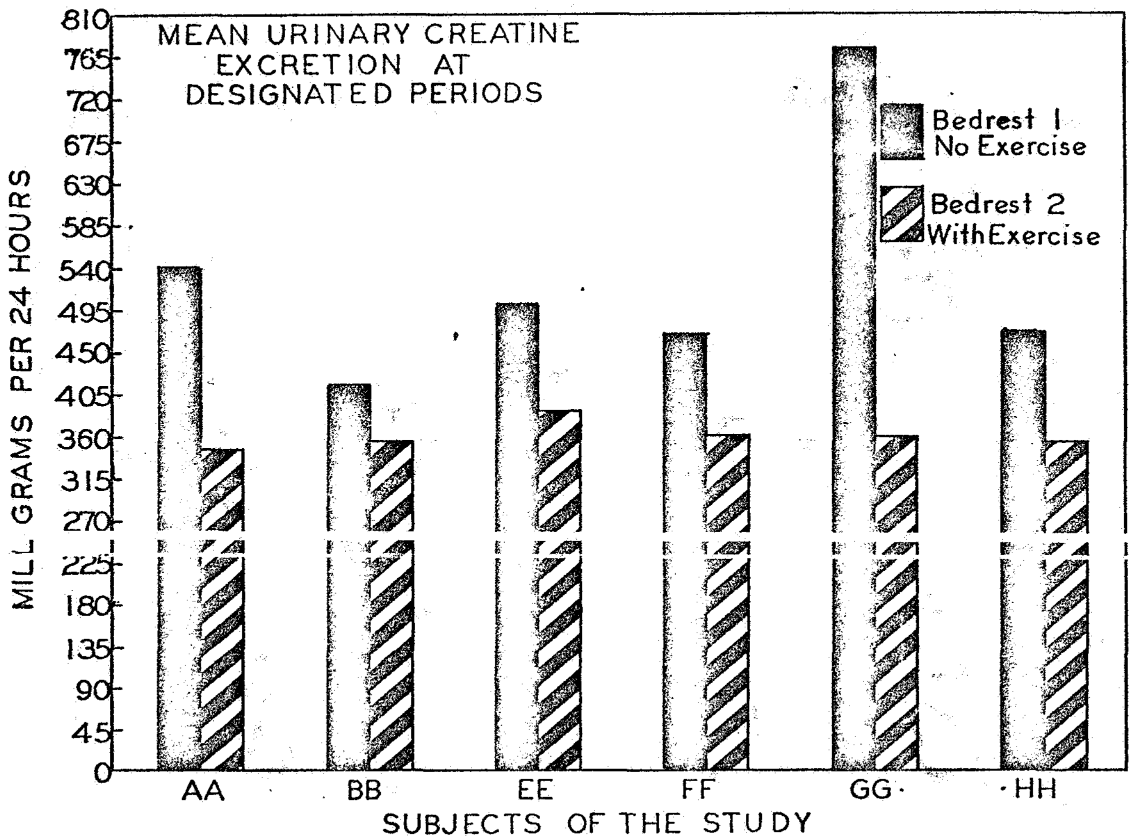


Figure 9. COMPARISON OF URINARY EXCRETION OF CREATINE DURING BED REST I (NO EXERCISE) AND BED REST II, WITH VARYING DEGREES OF EXERCISE FOR THE DIFFERENT SUBJECTS

Figure 10. BED REST SUBJECT EXERCISING WHILE COMPLETELY RECUMBENT IN HORIZONTAL BED REST

Monthly Report 19 June, 1969

Under Contract NAS 9-8246

SUBJECT: CIRCADIAN RHYTHM PATTERN OF 17-HYDROXY-CORTICOSTEROID URINARY EXCRETION IN HEALTHY ADULT HUMAN MALES

In recent years, many investigations have been conducted in order to find whether or not the adrenal cortex has a cyclic urinary excretion pattern within a 24 hour period. Pincus(40) first proposed a circadian rhythm of the adrenal cortical secretion in his study on the estimation of urinary 17-Ketosteroids. Apparently one of the major adrenal cortical hormones, cortisol, has been shown to have a clear circadian rhythm reflected both in the plasma concentration and urinary excretion of 17-hydroxycorticosteroids(29).

Several authors already have generalized concerning the fact that, under normal conditions, the maximum value of plasma 17-hydroxycorticosteroid was observed early in the morning, with the minimum noted during the late evening hours. The circadian rhythm of the urinary excretion of 17-hydroxycorticosteroids has a similar pattern to that of the plasm 17-hydroxycorticosteroid concentration, although the peak value usually was delayed two or three hours (12).

In the present study, the diurnal variation of urinary 17-hydroxycorticosteroids in normal healthy young men was investigated by measuring the amounts of urinary excretion of 17-hydroxycorticosteroids at different times of the day during conditions of ambulation and bed rest immobilization.

The Reddy method was used for the determination of urinary 17-hydroxycorticosteroids, which is based on the theory of color formation by the reaction of phenylhydrazine-sulfuric acid reagent on the dihydroxyacetone portion of 17-hydroxycorticosteroids(42)(43).

The main objectives of the present study were the following:

1. To study the effects of bed rest immobilization by healthy adult men on the urinary excretion of 17-hydroxycorticosteroids;

2. To study the circadian rhythm response of the adrenal cortex by measuring the urinary excretion of 17-hydroxycorticosteroids at various times during the 24-hour day; and

3. To investigate whether or not bed rest immobilization affects the circadian rhythm of urinary 17-hydroxycorticosteroids excretion in comparison with the ambulatory period.

PRESENTATION OF FINDINGS

The data of total daily urinary excretion of 17-hydroxycorticosteroids for the Initial Equilibration, Bed Rest, and Final Ambulatory Periods are presented in Table I (Appendix). Table II gives the data concerning the diurnal pattern of urinary 17-hydroxycorticosteroid excretion during the Bed Rest and Ambulatory Periods. For this purpose, the urine for each individual was pooled for the previous period at three different times of the day; 8:00 A.M., 12:00 Noon, and 8:00 P.M.. Table III presents the statistical analysis of daily urinary excretion of 17-hydroxycorticosteroids during the Equilibration, the Bed Rest, and the Final Ambulatory Periods comparing all periods to one another. Table IV gives the statistical findings comparing the urinary data collected during the three different periods of the day, both during Bed Rest and the following Ambulatory Periods.

COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROIDS EXCRETION DURING BED REST AND THE INITIAL EQUILIBRATION PERIOD

As shown in Figure 6, the average daily urinary excretion of 17-hydroxycorticosteroids during the Pre-Bed Rest and the Bed Rest

Periods varied among individual subjects. Somewhat higher excretion values of urinary 17-hydroxycorticosteroids occurred for subjects HH, AA, and EE during bed rest than during the Equilibration Period, while the subjects BB, FF, and GG had a slightly lower excretion of urinary 17-hydroxycorticosteroids during Bed Rest.

Comparing the data on an individual basis, the difference between the two periods was not statistically significant for any of the subjects except subject AA for whom the difference was only slightly significant ($P<0.10$).

When the data for all six subjects were pooled, slightly higher excretion values occurred during the Bed Rest Period as compared to the Pre-Bed Rest Period; but the difference was not statistically significant.

COMPARISON OF URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS DURING THE BED REST AND DURING THE FINAL AMBULATORY PERIOD

The daily amount of 17-hydroxycorticosteroids excreted in the urine during the Bed Rest and the Final Ambulatory Period are shown in Figure 6. The difference between these two periods were not statistically significant for each individual subject analyzed separately except for subject BB, whose excretion value for this steroid was significantly higher during the Final Ambulatory Period ($P<0.05$). When the data for all subjects were pooled, there was no statistically significant difference between the excretion of 17-hydroxycorticosteroids between these two periods.

COMPARISON OF URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS DURING THE EQUILIBRATION PERIOD WITH THE FINAL AMBULATORY PERIOD

The data of all six subjects pooled together showed no statistically significant difference in the amount of 17-hydroxycorticosteroids excreted in the urine during the initial and final ambulatory periods. On an individual basis, Subject BB was significantly higher during the Final Ambulatory Period than during the Initial Equilibration Period ($P<0.05$). This was the only subject in the study who showed this difference.

OVERALL COMPARISONS OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION PER DAY DURING THE PERIODS OF INITIAL EQUILIBRATION, BED REST, AND FINAL AMBULATION

As reported in the study of Vallbona, et. al. already cited (53), a slightly increased excretion of urinary 17-hydroxycorticosteroids for the average value of six subjects were found during the Bed Rest as compared with the Pre-Bed Rest Periods, even though there were variable individual differences. In the current study, again slightly increased values were recorded during the Final Ambulatory Period as compared both with the Bed Rest, and the Pre-Bed Rest Periods. All of these differences, however, were not statistically significant (Figure 6). According to these results, it may be stated that, in human beings, the change in urinary excretion of 17-hydroxycorticosteroids by immobilization does not occur in a relatively short time, unlike the animal cases reported by Burstein et. al. (6), which showed a two- to three-fold increase in corticosteroid excretion during immobilization.

COMPARISON OF EXCRETION OF 17-HYDROXYCORTICOSTEROIDS AT SUB-PERIOD OF THE 24-HOUR DAY

BED REST COMPARISONS

In order to ascertain whether or not there was any degree of rhythmicity in the urinary excretion of 17-hydroxycorticosteroids in certain segments of the 24-hour day, all voids between three daily periods were combined for analytical purposes. These were the periods from 8:00 A.M. until 12:00 noon, from 12:00 noon to

8:00 P.M., and from 8:00 P.M. to 8:00 A.M. Comparisons were made within the Bed Rest and the Final Ambulatory Periods. See Table IV.

For the data from all subjects pooled together for the Bed Rest Period, the sub-period from 8:00 A.M. until 12:00 noon had the highest level of excretion of this steroid (see Table IV), with the period from 12:00 noon to 8:00 P.M. ranking lowest, and that from 8:00 A.M. to 8:00 P.M. intermediate. This is shown also in Figure 7.

The morning period (8:00 A.M. to 12:00 noon) surpassed that excreted during the night and again that excreted from 12:00 noon until 8:00 P.M. by highly significant differences ($P<0.001$ in both cases). Again the 12:00 noon until 8:00 P.M. excretion exceeded the night excretion by a highly significant difference ($P<0.001$).

The individual subjects varied greatly as to the comparison of the excretion of the steroid under consideration during the three sub-periods of the 24-hour day. For four subjects there was no statistically significant difference between the 12:00 noon until 8:00 P.M. excretion as compared with that from 8:00 P.M. to 8:00 A.M., although the afternoon sub-period surpassed the night sub-period to some extent in all of these four subjects. One subject was superior in the afternoon sub-period by a highly significant difference ($P<0.001$), while for the other subject, the afternoon sub-period exceeded the night sub-period by a slightly significant difference.

In the comparison of the morning excretion with that of the afternoon, four subjects were significantly superior during the former by different levels of probability, whereas the remaining two were superior in the same direction, although the differences in the two cases were not statistically significant.

When the morning excretions of urinary 17-hydroxycorticosteroids were compared with the 12-hour night excretion, the former surpassed the latter in five of the six subjects of the study. In the sixth subject, the morning excretion of this steroid surpassed the night excretion, although the difference was not statistically significant.

FINAL AMBULATORY PERIOD COMPARISONS

For the data of all subjects pooled together, the sub-periods within the Final Ambulatory Period showed marked differences in some but not in all comparisons. The urinary excretion of 17-hydroxycorticosteroids during the morning sub-period from 8:00 A.M.

until 12:00 noon surpassed both the 12:00 noon until 8:00 P.M. period and the night sub-period ($P<0.001$ in both comparisons). There was no significant difference, however, between the 12:00 noon until 8:00 P.M. and the 12-hour night period, although the former was somewhat higher than the latter.

With respect to individual subjects, all six followed the overall pattern of all data pooled in that there was no statistically significant difference between the excretion of the steroid under consideration during the afternoon and the night sub-periods.

With respect to the comparison of the morning and the afternoon sub-periods during the Final Ambulatory Period, five subjects had statistically significant differences between the two portions of the 24-hour day in behalf of the morning period, with the sixth subject showing no significant difference in this respect.

In the comparison between the morning and the night sub-periods, again five subjects showed a significantly higher excretion of 17-hydroxycorticosteroids during the morning phase of the study with one subject not exhibiting a statistically significant difference between the two sub-periods.

OVERALL COMPARISONS OF URINARY 17-HYDROXYCORTICOSTEROIDS EXCRETION DURING THE BED REST AND FINAL AMBULATION PERIODS

The results obtained in the present study conform with those found in many previous studies in which a marked circadian rhythm of the 17-hydroxycorticosteroids was demonstrated. As noted above, the highest excretion of 17-hydroxycorticosteroids in the urine occurred in the morning hours (8:00 A. M. to 12:00 noon), with a far greater excretion during this period than during the other two sub-periods of the day.

Relatively low values were observed in the afternoon periods (12:00 noon to 8:00 P. M.), with the lowest generally recorded during the overnight period. The difference between the latter two periods was not statistically significant for the pooled data of all subjects.

Similar diurnal patterns of urinary 17-hydroxycorticosteroid excretion were obtained during the ambulatory periods. This also is identical with the report of Katz (24).

Thus, according to these findings, it may be stated that the biological cycle of adrenal cortex which is reflected on the urinary excretion of 17-hydroxycorticosteroids is relatively unchangeable by the mild stress condition of immobilization.

SUMMARY AND CONCLUSION

The present study was designed to investigate the effects of bed rest immobilization on the total amount of urinary 17-hydroxycorticosteroids excreted, and the circadian rhythm pattern of this steroid during the 24-hour day. The experimental periods of the study consisted of three phases: 29 days of initial equilibration during which the subject was ambulatory and led a normal life; 28 days of horizontal bed rest, which prohibited physical movement which involved pull on the muscles; and 14 days of ambulation which included compulsory physical activity.

Although there were some variations among the individuals of the study, the data of all subjects pooled together showed that there were no statistically significant differences between the total 24-hour excretion of 17-hydroxycorticosteroids during the Equilibration Period and the Bed Rest Period, between the Bed Rest Period and the Final Ambulatory Period, or between the two ambulatory periods.

A marked characteristic circadian cycle was observed during bed rest. It was found to have its highest excretion value during the morning hours (8:00 A.M. to 12:00 noon), a low excretion value during the afternoon period (12:00 noon to 8:00 P.M.), and the lowest values during the overnight period (8:00 P.M. to 8:00 A.M.). The differences of average values between those experimental periods were as follows: a highly significant difference appeared between the morning and afternoon periods, as well as a highly significant difference between the period of morning and overnight, and again a highly significant difference between the afternoon period and the overnight period.

During the final ambulatory period, the overall phase of diurnal rhythm of urinary 17-hydroxycorticosteroids was found to be similar to that during the bed rest, although a somewhat different feature was noted between the periods of afternoon and overnight. No statistically significance was found in the latter comparison as compared with the highly significant difference between these two periods during bed rest. The data arranged by statistical analysis during this ambulatory period were obtained as follows: highly significant difference ($P<0.001$) in urinary 17-hydroxycorticosteroids excretion between the periods of morning and afternoon, also highly significant difference ($P<0.001$) between afternoon period and overnight periods, and no statistically significant difference between the periods of afternoon and overnight.

URINARY 17-HYDROXYCORTICOSTEROIDS
DURING BED REST I
(Mean Milligrams Per Hour)